Sodium Citrate Stabilized Calcium Phosphate Nanoparticles for the Sustained Delivery of Cisplatin

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Statement of Purpose: Calcium phosphate (CaP) nanoparticles (nanoCaP) are an attractive vehicle for the delivery of anti-cancer compounds due to their biocompatibility, low cost, and ease of manufacture. Drug delivery vehicles offer a means of reducing anti-cancer side effects. To produce nano-sized (< 300 nm) CaP particles, a stabilizer must added to halt CaP crystal growth and avoid agglomeration. The stabilizer must not interfere with the released therapeutic activity nor be toxic. Here we describe the manufacture, characterization, and *in vitro* and *in vivo* efficacy of sodium-citrate (citrate) stabilized nanoCaP which carry cisplatin (CDDP), a commonly used chemotherapeutic. The following hypotheses were tested: if sodium citrate is a successful stabilizer then 1) citrate added to CaP with CDDP will be nano-sized, 2) citrate nanoCaP with CDDP will be cytotoxic *in vitro*, and 3) citrate nanoCaP with CDDP will demonstrate efficacy inhibiting tumor growth *in vivo* without any observed significant drug toxicity (weight loss).

Methods: NanoCaP production was based on a previously reported method except a new stabilizer molecule was used, sodium citrate [1]. Equal volumes of 30 mM $CaCl_2$ and 30 mM K_2HPO_4 + 20 mM citrate were mixed. Nanoparticles were collected and allowed to bind for 20 hrs in aquated cisplatin solution (Aq-CDDP). Following binding the particles were collected, washed, and diluted with 20 mM citrate solution to make an injectable suspension. CDDP content was determined by inductively coupled plasma-optical emission spectroscopy (Perkin Elmer® Optima™ 5300 DV, ESIS Inc., Cromwell, CT). Particle size analysis (PSA) was performed using a 90 Plus Particle Sizer (Brookhaven Instruments, NY). Cytotoxicity experiments were conducted using FaDu human hypopharyngeal carcinoma cells in an MTT assay (CellTiter 96® AQ_{ueous} One, G3580, Promega Corp., Madison, WI)) to determine the IC_{50} (50% inhibitory concentration) of Aq-CDDP, 20 mM citrate aquated cisplatin (Cit-Aq-CDDP), particles without citrate (CaP/CDDP), and nanoCaP-cit/CDDP. Statistical significance was determined by one way ANOVA with Tukey post test. For the *in vivo* study, Nu/J mice were injected with $2x10^6$ FaDu cells subcutaneously. Tumors were treated once, intratumorally, with either 30 µL of saline, 20 µL of nanoCaP-Cit (without CDDP), 1.4 mg/kg cisplatin in saline (CDDP IT), or 10 mg/kg nanoCaP-Cit/CDDP, when tumor volume reached 140±14 mm^3. Tumor volume and mouse weight were monitored daily. Significant weight loss was considered > 15%. Statistical significance between groups was based on Tukey one-way ANOVA.

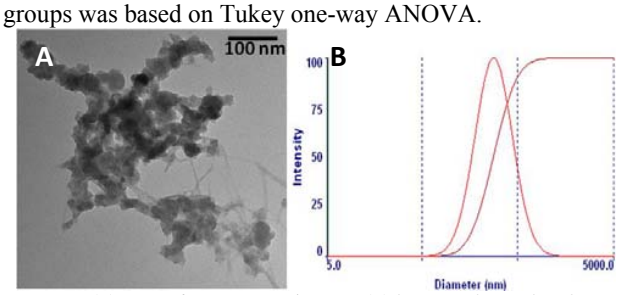

Figure 1: *(A) TEM of nanoCaP-cit/CDDP, (B) lognormal PSA distribution, average particle size of 281.3±27.5 nm.*

Results: Transmission electron microscopy (TEM) and particle size analysis confirmed that citrate nanoCaP with CDDP were nano-sized: 281.3±27.5 nm, (Fig. 1). Drug loading of the suspension was 154.9 µg CDDP/mg cit-CaP. Cytotoxicity testing revealed that citrate was not cytotoxic to the FaDu cell line, when used at the same concentration in nanoCaP-cit/CDDP, (Fig. 2A). However, Cit-Aq-CDDP had decreased cytotoxicity compared to Aq-CDDP, IC_{50} values of 35.47 µM vs. 11.30 µM respectively, (Fig. 2A). Sodium citrate significantly decreased the cytotoxicity of the particles compared with those with no stabilizer, nanoCaP-Cit/CDDP (IC_{50} = 22.63 µM) vs. CaP/CDDP (IC_{50} = 15.88 µM), (P < 0.5), data not shown.

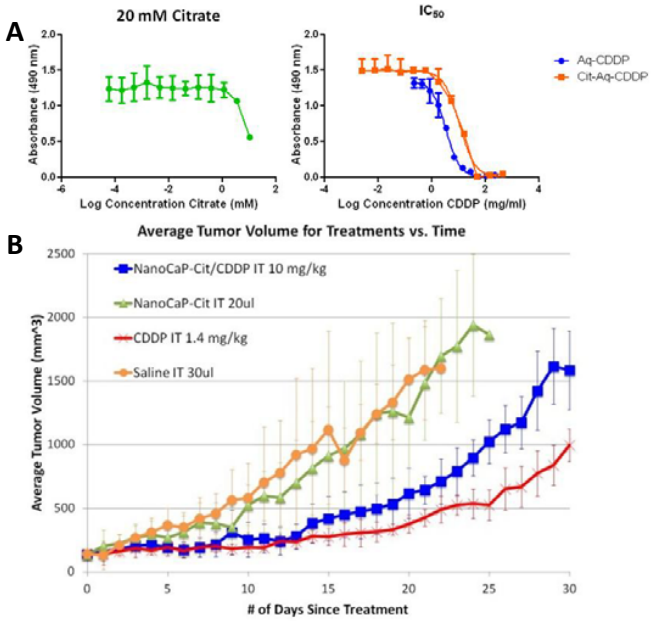

Figure 2: *(A) IC_{50} curves for 20 mM citrate, Aq-CDDP (11.30 µM), Cit-Aq-CDDP (35.47 µM), (B) in vivo average tumor volume.*

Efficacy of nanoCaP-Cit/CDDP was demonstrated in the *in vivo* FaDu tumor model, (Fig. 2B). 10 mg/kg nanoCaP-Cit/CDDP resulted in delayed tumor growth. There were significant differences between Saline and 10 mg/kg nanoCaP-Cit/CDDP, Saline and CDDP, and 10 mg/kg nanoCaP-Cit/CDDP and CDDP, (P < 0.5). No significant weight loss was observed for any of the treatment groups.

Conclusions: Citrate stabilized calcium phosphate cisplatin particles are cytotoxic and inhibit tumor growth; however cisplatin alone was most effective *in vitro* (Aq-CDDP) and *in vivo* (CDDP IT). These results are most likely due to citrate interference with cisplatin activity. Additional stabilizers for calcium phosphate need to be identified that do not interfere with cisplatin activity in order to improve upon cisplatin cytotoxicity and *in vivo* efficacy.

References: [1] Cheng X. and Kuhn LT. Int. J. of Nanomedicine. 2007:2(4):667-674.