Efficacy of Novel Active Targeting Dendrimer for Paclitaxel Delivery to Breast Cancer Cells

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Purpose: Breast cancer is the 2nd most common cause of cancer-related deaths in women worldwide. Chemotherapy is an important treatment modality. Yet, delivery of drugs such as the chemotherapeutic paclitaxel (PTX) to cancer cells remains a challenge, mainly due to a drug's low solubility, rapid *in vivo* clearance, high systemic toxicity, and low drug delivery to tumor. Nanotechnological devices have been proposed as a solution to these problems. Increased tumor specificity of nanoparticles is achieved through attachment of agents that target the molecular differences between normal and malignant cells, a process known as active targeting. Dendrimers are a category of synthetic, monodisperse and multivalent nanopolymers, capable of high drug-loading and multiple covalent bonding of bioactive moieties on the periphery. Conjugation using enzyme cleavable linkers allows for specific, targeted release. While PTX delivery through dendrimers has been established,[1] such vehicles have not used active targeting for specific delivery to cancer cells. Thus, a need exists for specific delivery of PTX through an active-targeting dendrimer-based vehicle, while maintaining high-drug loading properties of dendrimers. This study reports the synthesis, characterization, and *in vitro* assessment of polyamido-amine dendrimers conjugated to PTX via a peptide spacer cleavable by a cancer-upregulated enzyme, Cathepsin B (CatB).

Methods: *Synthesis of PGD Dendrimer:* Polyamidoamine (PAMAM, Generation 4; Sigma) was conjugated to PTX (ChemieTek) via an enzyme-cleavable, peptide spacer. Briefly, PTX was converted to its hemisuccinate by a reaction with succinic anhydride. Following purification and activation, an equimolar mixture of activated PTX hemisuccinate and the CatB substrate tetrapeptide glycine-phenylanaline-leucine-glycine (GFLG; Biomatik) was reacted anhydrously in the presence of a base. The purified and activated PTX-GFLG conjugate was finally reacted to PAMAM at a 16:1 molar ratio to form the PTX-GFLG-dendrimer product, referred to as PGD. Structure confirmation of PGD was done by ^{1}H-NMR, reversed phase HPLC, and mass spectrometry.

Cell culture: MDA-MB-231 (MM231) human breast cancer cells and MCF10A immortalized normal breast cells were cultured in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic at 37ºC with 5% CO_2. Cells were maintained in culture flasks with media changes every 2 days and passaged using trypsin prior to experimentation.

Cytotoxicity assay: Cells (2 x 10^3 cells/well) were seeded in 100 µL culture medium in 96-well culture plates and were incubated for 24h. Previous media was aspirated and replenished with fresh RPMI 1640 culture medium supplemented with 5% FBS and 1% antibiotic/antimitotic and containing various concentrations of PTX or PTX-equivalent dendrimer (0.01 nM to 1 µM). Media alone served as a blank and plain PAMAM at equimolar concentration was the negative control. After 72h, 10 µL of WST-1 Cell Proliferation Reagent (Roche Diagnostics) was added to each well and plates were incubated for an additional 4h. Absorbance of the converted WST-1 product was read at 415 nm on a microplate reader (Synergy 2, Biotek). The relative IC_{50} data were calculated by GraphPad Prism 5 (GraphPad Software).

Results: Synthesis verification by time-of-flight mass spectrometry yielded a broad product peak centered on a molecular weight of ~24 kDa. NMR proton integration revealed a PTX to dendrimer ratio of approximately 7:1. This ratio was used to create dilutions of PGD to deliver specific molar amounts of PTX in cell assays.

Cytotoxicity assays using WST-1 reagent for the breast cancer cell line MM231 demonstrated a marked shift in the relative IC_{50} values of PTX incorporated in PGD (Fig 1a). The relative IC_{50} concentration is the half-way point between the top and bottom plateaus of the compound concentration curve.[2] The IC_{50} of paclitaxel for MM231 cancer cells, a cell line with a highly upregulated CatB activity,[3] was 0.640 nM for PGD relative to 3.29 nM for PTX. By contrast, the IC_{50} of for MCF10A normal cells, having a low/normal CatB activity, was 2.45 nM for PGD as compared to 2.85 nM for PTX. This smaller shift in relative IC_{50} values correlates to reported CatB activity. When compared at 10 nM concentration alone (Fig 1c), negative control PAMAM had no effect on cell growth, while PGD had a statistically significant difference compared PTX for MM231 cells only

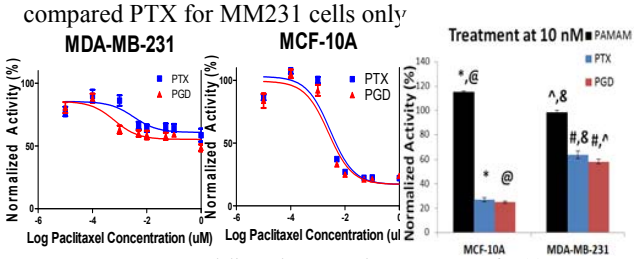

Figure 1. PTX conc. delivered as PTX alone or as PGD for (a) MDA-MB-231 and (b) MCF10A cells. (c) PTX at 10 nM delivered as PTX alone, PGD, or blank (PAMAM) (ANOVA/Tukey, $p<0.05$ marked).

Conclusions: PTX when delivered through PGD showed increased cytotoxicity to MM231 cancer cells but not normal MCF10A cells, correlating with previous reports on CatB activity. This suggests successful development of a cancer-specific active targeting delivery vehicle.

References: **1**. Torchilin VP. Nanoparticulates as Drug Carriers 2006; p287-289. **2.** GraphPad, http://www.graphpad.com/support/faqid/1566/ **3.** Krepela E, et al. Neoplasma 1989; 36:41-52.

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