

Development of hydrophobic taxane prodrug nanoparticles with enhanced plasma circulation lifetime and improved efficacy

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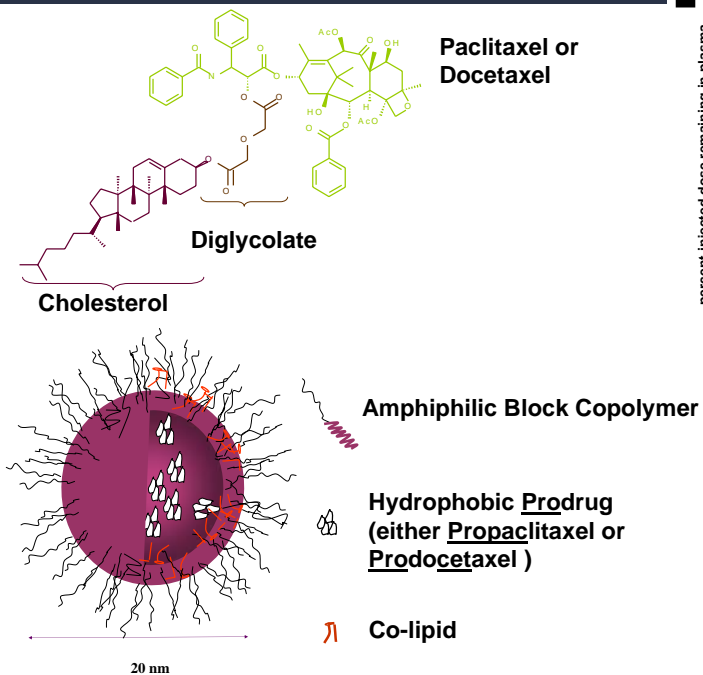
Abstract 3698



INTRODUCTION

Drug carriers can alter the pharmacokinetics and biodistribution of drugs relative to the free agents. Nanoparticles formed using amphiphilic diblock co-polymers can be used as drug carriers for hydrophobic chemotherapeutic agents, however block co-polymer nanoparticles frequently display burst release of trapped agents upon injection into animals. As a result, these nanoparticles often show only limited improvements in drug circulation lifetime and likely provide little improvement in drug delivery to the tumor site. To address the issue of formulation stability, we have generated hydrophobic prodrugs of taxanes to enhance retention within the nanoparticle. We observed a correlation between drug circulation lifetime of paclitaxel prodrugs and the hydrophobicity of the anchor. When these formulations were evaluated for anti-tumor efficacy, the longest circulating paclitaxel prodrugs were the most efficacious. We have now extended our assessment to hydrophobic docetaxel prodrug formulations and have related plasma circulation characteristics of the docetaxel prodrug nanoparticles to that of paclitaxel prodrug nanoparticles. We also compared the suppression of tumor growth in mice bearing HT-29 human colorectal tumor xenografts. Cholesterol-anchored prodrugs, Propac 8 and Procet 8 were formulated in PS-PEG nanoparticles. The plasma half-life of the docetaxel prodrug and the corresponding paclitaxel prodrug were similar, however the improved efficacy of the prodrug over the respective free drug was significantly greater for the docetaxel prodrug. These results confirm that hydrophobic docetaxel prodrugs can be formulated into block co-polymer nanoparticles to achieve prolonged circulation kinetics which result in enhanced therapeutic activity.

SCHEME FOR PROPAC AND PROGET NANOPARTICLES

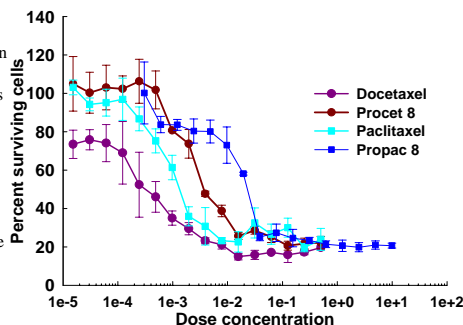


Nanoparticles are comprised of amphiphilic polystyrene-block-polyethyleneglycol (3K:2.5K), co-lipid (1-palmitoyl-2-oleoyl phosphatidylcholine) and prodrugs. These particles are generated by rapidly mixing components dissolved in solvent with water using a jet impinging mixer¹. Drug-containing nanoparticles retain drug and maintain their size when stored in water or sucrose for several months at 4°C.

1. Ansell et al. Modulating the therapeutic activity of nanoparticle delivered paclitaxel by manipulating the hydrophobicity of PTX-prodrug conjugates. J. Med. Chem. 2008, 51(11):3288-96.

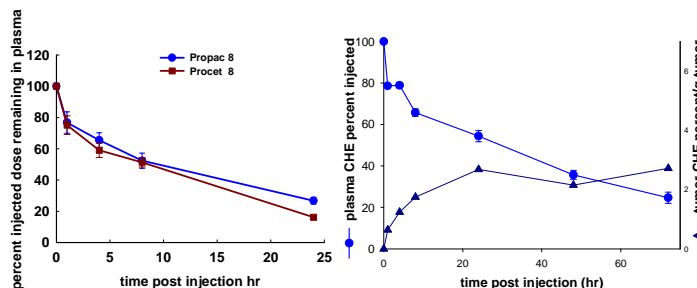
RESULTS

1. CYTOTOXIC ACTIVITY OF TAXANE PRODRUGS CAN BE RECOVERED IN VITRO



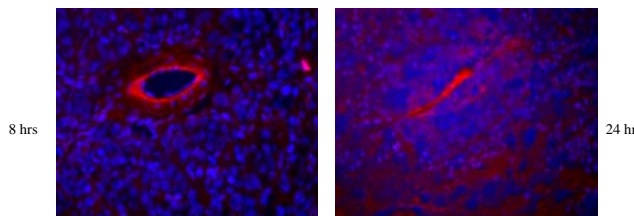
Propac 8 and Procet 8 were cytotoxic to MCF7 cells. Cells were incubated with prodrugs or parent drugs for 3 days and the surviving cell fraction was evaluated using the MTT cytotoxicity assay. We have previously observed cytotoxic activity with prodrugs which are readily hydrolysed to parent drugs, while agents which are not hydrolysed are relatively inactive (data not show). We postulate that the observed activity indicates that Propac 8 and Procet 8 regenerate parent agents when incubated with cells.

2. LONG CIRCULATING NANOPARTICLES ACCUMULATE AT THE TUMOR SITE



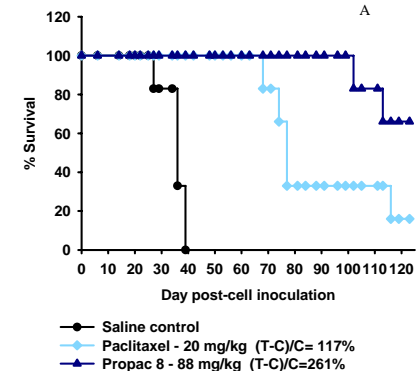
Propac 8 and Procet 8, formulated in nanoparticles were injected i.v. into Foxn-1 mice. The plasma levels of Propac 8 and Procet 8 were monitored for 24 hours. The plasma concentration correlates with the retention of the drug conjugate by the nanoparticle demonstrating that nanoparticles are stable to injection and are long circulating.

A hydrophobic radioactive marker, Cholesteryl-hexadecyl ether (CHE) remains trapped in nanoparticles incubated in serum (data not shown). Propac micelles prepared with CHE were injected into HT-29 tumor-bearing Foxn-1 mice. The plasma CHE levels and the CHE accumulation in tumors at various times after injection are shown above. Nanoparticles accumulate in tumors for up to 24 hours.

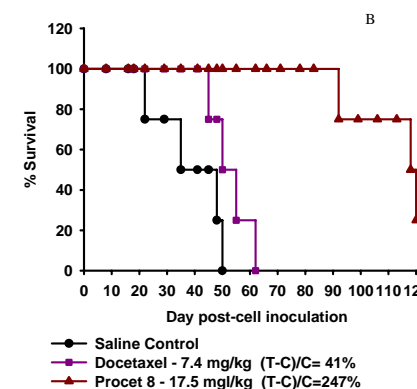


Tumor bearing mice were injected with nanoparticles formed with a lipophilic carbocyanine dye, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine or DiD. DiD-labelled nanoparticles localization within HT 29 tumors was determined at 2 time points. Tumor slices were counterstained with DAPI to highlight cellular localization. Nanoparticles are observed close to the blood vessel after 8 hours but redistribute throughout the tumor by 24 hours.

3. EFFICACY OF TAXANE PRODRUG NANOPARTICLES IS INCREASED RELATIVE TO FREE DRUGS AT MTD



Mice bearing HT29 solid tumors were treated at maximum tolerated dose of paclitaxel prodrugs formulated in nanoparticles (A) or docetaxel prodrugs in nanoparticles (B) on a Q2DX5 dosing schedule. Dosing began when subcutaneously implanted tumors reached size of 80-120 mg (day 14). Propac 8 and Procet 8 significantly prolonged the survival of tumor-bearing mice. Propac 8 and Procet 8 at MTD were more active than the corresponding free drug in prolonging survival. A greater improvement observed with Procet 8



Animals were euthanized when tumors reached 1000mg. Survival was calculated when population declined to 66% original size.

SUMMARY

- Paclitaxel (Propac) and Docetaxel (Procet) prodrug-conjugates were long circulating in nanoparticle formulations
- In vitro cytotoxicity suggests that the active parent drug can be regenerated
- Optimized Propac and Procet nanoparticles displayed therapeutic activity in the HT-29 solid tumor model that was superior to that of the parent drug
- The relative anti-tumor activity of the parent compound does not predict the activity of the formulated prodrug