Use of PBMC Partitioning to Predict RES-Uptake of a Nanoemulsion in a Colon Cancer Xenograft

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Nanotechnology platforms have emerged as versatile carrier systems for delivery of active molecules to tumors. Nanoparticle-based formulations have demonstrated enhanced efficacy and decreased toxicity in comparison to conventional drugs. These carrier systems often encounter rapid uptake by phagocytic cells of the reticuloendothelial system (RES), which is a major obstacle to tumor accumulation. Experimental models are required that can predict RES sequestration, and allow for optimization of nanoparticle properties, such as size, surface charge, and PEGylation.

Purpose: The aim of the present work was to determine the applicability of using peripheral blood mononuclear cell (PBMC) partitioning of nanoparticles as an accessible surrogate for monitoring of RES distribution.

Methods: Paclitaxel (PTX) loaded o/w emulsion was prepared by using Microfluidizer® - processor M-110EH (Fig.1A). Briefly, an aqueous homogenous dispersion was prepared in deionized water and egg phosphatydylcholine, added to a flaxseed oil phase, and finally passed through a micro fluidizer to generate a uniformly distributed nanoemulsion. PBMC distribution of the novel PTX nanoemulsion and two commercial PTX formulations (Taxol ™ and Abraxane®) were evaluated in whole mouse blood *in vitro*. The pharmacokinetics of the novel PTX nanoemulsion was then further evaluated *in vivo*, characterizing plasma, PBMC, tumor and RES organ pharmacokinetic profiles in a colon cancer xenograft (LS174T). After reaching a tumor size of 8mm dia., a single 18.4mg/kg dose of the PTX nanoemulsion was administered by tail vein. Blood, liver, spleen and tumor were collected at 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 24h time points. Plasma was separated from whole blood by centrifugation, and the PBMC fraction was isolated by the Ficol-paque gradient method. PTX concentrations in Blood, plasma, monocytes and tissues were determined by a validated HPLC method. Pharmacokinetic parameters were determined using WinNonlin version 4.1 software (Pharsight, Mountain View, CA).

Results: PTX-loaded nanoemulsion droplets were reproducibly prepared by the high pressure homogenization method. Blank and drug-containing nanoemulsion droplets had Z-average diameters of 160 and 180 nm, respectively, and corresponding zeta potentials of +70.9 and +73.0 mV (Fig.1B). During 4h incubation in whole mouse blood, increased PTX PBMC fractioning (~2 folds) was observed with nanoemulsion in comparison to Taxol ™ and Abraxane ™. The pharmacokinetic profile of PTX nanoemulsion in Blood, plasma, liver and spleen followed biphasic decays, with the PBMC fraction and tumor following monophasic decays (Fig.2A). In agreement with the in vitro PBMC uptake studies, greater distribution of PTX was found in liver, PBMC and spleen in comparison to Blood, plasma and tumor mass (Fig.2A&B).

Conclusions: These data support the hypothesis that uptake into the PBMC fraction *in vitro*, and *in vivo*, can be used to estimate distribution of nanoformulations to RES organs.

Acknowledgements: This study was supported by the Nanotechnology Platform Partnership grant (R01-CA119617) and NCI Contract HHSN261200800001E.

Figure 1

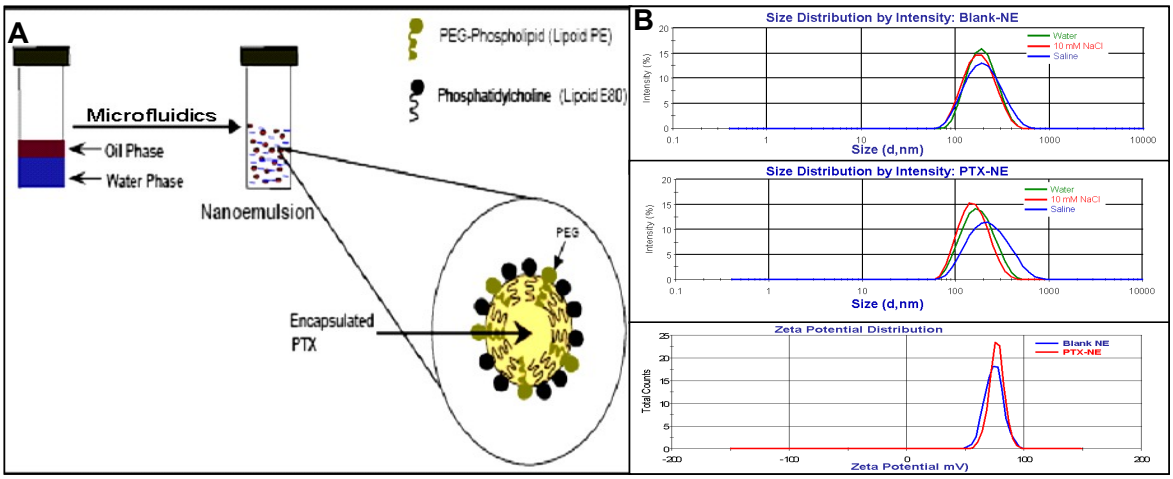

Figure 2