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Profiling the relationship between tumor-associated macrophages and pharmacokinetics of liposomal agents in preclinical murine models

Andrew T. Lucas, PharmD, MS[a], Taylor F. White, BS[b], Allison M. Deal, MS[c], Leah B. Herity, BS[b], Gina Song, PhD[a], Charlene M. Santos, BA[d,e], William C. Zamboni, PharmD, PhD[a,b,d,*]

[a]*Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*

[b]*UNC Eshelman School of Pharmacy, Chapel Hill, NC, USA*

[c]*Lineberger Comprehensive Cancer Center Biostatistics Core, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*

[d]*Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*

[e]*The Animal Studies Core, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*

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Abstract

The mononuclear phagocyte system (MPS) has previously been shown to significantly affect the clearance, tumor delivery, and efficacy of nanoparticles (NPs). This study profiled MPS cell infiltration in murine preclinical tumor models and evaluated how these differences may affect tumor disposition of PEGylated liposomal doxorubicin (PLD) in models sensitive and resistant to PLD. Significant differences in MPS presence existed between tumor types (e.g. ovarian versus endometrial), cell lines within the same tumor type, and location of tumor implantation (i.e. flank versus orthotopic xenografts). Further, the differences in MPS presence of SKOV-3 ovarian and HEC1A endometrial orthotopic cancer models may account for the 2.6-fold greater PLD tumor exposure in SKOV-3, despite similar plasma, liver and spleen exposures. These findings suggest that profiling the presence of MPS cells within and between tumor types is important in tumor model selection and in tumor types and patients likely to respond to NP treatment.

Key words: Mononuclear phagocyte system; Tumor-associated macrophages; PEGylated liposomal doxorubicin; Preclinical cancer models; Nanoparticles

Liposomal drug delivery systems have been studied extensively to increase the solubility and therapeutic index of chemotherapeutic agents.[1] A variety of agents have been implanted into liposomes because their biological properties are attractive, including improved solubility of hydrophobic compounds, increased stability of large molecules, improved efficacy, and reduced toxicity.[2] After administration, unlike small-molecule drugs, the distribution of liposomes is greatly

Abbreviations: AUC, area under the curve; CMA, carrier-mediated agent; EPR, enhanced permeation and retention; GEMM, genetically engineered mouse model; HPLC-FL, high performance liquid chromatography-fluorescence; IHC, immunohistochemistry; IV, intravenous; KM, Kaplan–Meier; MPS, mononuclear phagocyte system; MTD, maximum tolerated dose; NP, nanoparticle; PD, pharmacodynamics; PK, pharmacokinetics; PLD, PEGylated liposomal doxorubicin; SC, subcutaneous; S-CKD602, PEGylated liposomal CKD602; SM, small-molecule.

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*Corresponding author at: Division of Pharmacotherapy and Experimental Therapeutics, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7361.

E-mail address: zamboni@email.unc.edu (W.C. Zamboni).

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limited because they are larger than the gaps in the endothelial walls of most normal tissues. However, tissues surrounded by the endothelial wall with larger gaps, such as liver, spleen, and bone marrow, usually are the major deposition sites of liposomes.[3,4] Moreover, tumor tissues have abnormal blood vessels and lack effective lymphatic drainage, which allows liposomes to enter and be retained in tumors. This phenomenon is called the enhanced permeation and retention (EPR) effect.[5,6]

As drugs are encapsulated in nanoparticles (NPs), such as liposomes, the pharmacokinetic (PK) disposition of these agents is dependent upon the carrier, and not the parent drug, until the drug is released from the carrier.[7,8] The PK of most NPs is more variable than small-molecule (SM) drugs. This was reported in a meta-analysis comparing differences in PK variability of liposomal and non-liposomal anticancer agents.[9] The PK variability contributes in part to variability in a drug's pharmacodynamic (PD) effects, making it difficult to predict how a particular patient will respond in terms of efficacy and/or toxicity.[10] This issue raises concern about the translational development and clinical utility of NP agents. In addition, these results highlight the need to identify the factors responsible for the PK and PD variability of these agents as a method to improve response and reduce toxicity.

Previous studies suggest that the significantly high and clinically relevant variability in the PK and PD of liposomal and NP anticancer agents is related to mononuclear phagocyte system (MPS) function, which serves as the clearance pathway for NP agents.[11-13] However, NP PK/PD variability between human patients can be attributed to many variables. These variables include duration of contact and overall activity of the MPS components, which are composed of circulating monocytes and dendritic cells and phagocytic cells in the liver and spleen. Previously, it has been reported that the variability in the PK/PD of NPs such as Doxil® (PEGylated liposomal doxorubicin; PLD) and S-CKD602 (PEGylated liposome of CKD-602, a camptothecin analog) was associated with patient age, gender, and the function of circulating monocytes in plasma of patients with solid tumors.[13-15] The relationship between the MPS and NP clearance has also been demonstrated in patients with refractory solid tumors.[16] This phase I study showed that unlike SMs, there was a bidirectional interaction between NPs and MPS cells, where the MPS cells are involved in the uptake and clearance of NPs. The uptake of NPs by MPS cells then alters the function of these cells.

Tumor exposure and antitumor activity of liposomal anticancer agents were also found to be related to the presence of the MPS in tumors, where increased delivery and release of drug from a liposomal agent were consistent with increased presence of MPS cells in SKOV-3 ovarian xenografts compared to A375 melanoma xenograft.[13] These results suggest that variability in the MPS may affect the tumor disposition and activity of liposomal anticancer agents.[13] It has been reported that there is significant heterogeneity in the microenvironment of tumors.[17,18] A recent study reported that heterogeneous tumor microenvironment and/or tumor cell features were associated with differences in the tumor delivery and efficacy of PLD, but not SM-doxorubicin, in GEMMs of triple-negative breast cancer.[19] These findings implicate that profiling of the tumor microenvironment and selection of patients with tumors conducive to NPs are required for the optimal delivery and therapeutic outcomes for NP-based therapy.

It has been unclear why within a patient with solid tumors there can be a reduction in the size of some tumors, whereas other tumors can progress during or after treatment, although the genetic composition of the tumors is similar.[20,21] Such variable antitumor responses within a single patient may be associated with inherent differences, such as in tumor vascularity, capillary permeability, and/or individual MPS infiltration. These differences result in variable delivery of anticancer agents to different tumor sites.[20,21] Similar effects may also be occurring in preclinical studies, and thus, studies need to be performed in preclinical tumor models to determine factors that alter tumor delivery of NPs.

The objective of the current study is to profile preclinical human tumor xenograft models (including ovarian, breast, endometrial, lung, and melanoma models) for MPS cells (i.e. macrophage presence) using immunohistochemistry (IHC). We also profiled the effect of the location of the xenograft implantation (flank versus orthotopic) on the altered characteristics of MPS cell infiltration within the same cell lines. In addition, we evaluated the effects of differences in the presence of macrophages on the PK and PD disposition of PLD and SM-doxorubicin in ovarian and endometrial cancer xenograft models. The results of these studies may help us in identifying the relationship between types of preclinical tumor models, MPS factors, and changes in the PK and PD of NPs.

Methods

Mice

All mice were handled in accordance with the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, 2011), and studies were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of North Carolina at Chapel Hill (Chapel Hill, NC). Mice (female CB-17 SCID, 5-6 weeks of age, and specific pathogen free) were obtained from Taconic Farms (Albany, NY) and were allowed to acclimate to the animal facilities at the University of North Carolina for 1 week prior to initiation of study. Body weights and tumor size were measured biweekly, and clinical observations were made twice daily.

Tumor lines

Human cancer cell lines were obtained from the UNC tissue culture facility via the American Type Culture Collection (ATCC; Rockville, MD): SKOV-3 (ovarian), ES-2 (ovarian), CAOV3 (ovarian), OVCAR3 (ovarian), MCF7 (breast), MDA-MB231 (breast), SUM149 (breast), BT-474 (breast), KLE (endometrial-endometrioid), RL95–2 (endometrial-endometrioid), HEC1A (endometrial-endometrioid), AN3CA (endometrial-endometrioid), A549 (lung), and A375 (melanoma). An additional endometrial-serous cell line, SPEC2, was kindly provided by Dr. Victoria Bae-Jump (University of North Carolina at Chapel Hill). Cell lines were authenticated using short tandem repeat profiling. Cells were expanded in culture to