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Research Paper

Pharmacokinetic and screening studies of the interaction between mononuclear phagocyte system and nanoparticle formulations and colloid forming drugs☆

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ABSTRACT

Studies have shown that nanoparticles (NPs) are cleared through the mononuclear phagocyte system (MPS). Pharmacokinetic studies of Doxil, DaunoXome, micellar doxorubicin (SP1049C) and small molecule (SM) doxorubicin were performed in SCID mice, Sprague-Dawley rats, and beagle dogs. An *ex vivo* MPS profiling platform was used to evaluate the interaction between the same agents, as well as colloid-forming and non-colloid forming SM drugs. In all species, the systemic clearance was highest for SP1049C and lowest for Doxil. With the exception of dog blood, the MPS screening results of mouse and rat blood showed that the greatest reduction in phagocytosis occurred after the *ex vivo* addition of SM-doxorubicin > SP1049C > DaunoXome > Doxil. The MPS profiling platform in rats, but not dogs, could differentiate between colloid forming and non-colloid forming drugs. The results of the MPS profiling platform were generally consistent with *in vivo* clearance rates of NP and SM anticancer drugs in mice and rats. This study suggests the MPS profiling platform is an effective method to screen and differentiate the important characteristics of NPs and colloid-forming drugs that affect their *in vivo* clearance. Implications of these findings on preclinical prediction of human clearance are discussed.

Abbreviations: ABC, accelerated blood clearance; ANC, absolute neutrophil count; BLQ, below limit of quantification; CL, clearance; DC, dendritic cells; DHR, dihydrorhodamine; FCM, flow cytometry; fMLP, *N*-formyl-methionine-leucine-phenylalanine; GLP, Good laboratory Practices; HPLC, high-performance liquid chromatography; LLOD, lower limit of detection; LLOQ, lower limit of quantification; MFI, mean fluorescence intensity; MO, monocytes; MPS, mononuclear phagocyte system; MTD, maximum tolerable dose; NP, nanoparticle; PEG, polyethylene glycol; PD, pharmacodynamics; PK, pharmacokinetic; PMA, phorbol myristate acetate; ROS, reactive oxygen species; SM, small molecule.

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1. Introduction

Nanoparticles (NPs), which include polyethylene glycol (PEG)-coated (PEGylated) liposomes, are novel drug delivery platforms that have the potential to improve tumor drug exposure and reduce accumulation in normal tissues compared with their small-molecule (SM) counterparts (Zamboni 2000, 2005, 2008). The biodistribution of NPs is dependent upon the carrier and not the drug encapsulated within the carrier until the drug gets released from the carrier (Zamboni 2005, 2008; Papahadjopoulos et al., 1991; Park et al., 2004). After the drug is released from the carrier, the pharmacokinetic (PK) characteristics of the drug are assumed to be the same as that following administration of the non-carrier form of the drug (Zamboni and Tonda, 2000; Zamboni, 2005, 2008).

Studies suggest that the significantly high and clinically relevant inter-patient variability in the pharmacokinetic and pharmacodynamic (PD) characteristics of NP anticancer agents is related to the function of monocytes and dendritic cells (MO/DC) of the mononuclear phagocyte system (MPS) (Zamboni et al., 2001, 2011). A study was previously published that demonstrates a significant relationship between the PK and PD of S-CKD602, a liposomal formulated camptothecin analog, and changes in circulating monocyte numbers and absolute neutrophil count (ANC) (Zamboni et al., 2011). The results of this study suggest that monocytes are more sensitive to toxic effects of S-CKD602 compared with neutrophils and that the increased sensitivity appears to be related to the liposomal formulation and not the SM drug, CKD-602, encapsulated within the liposome. Moreover, a recent study reported that the function (*i.e.* phagocytosis and production of reactive oxygen species (ROS)) of monocytes in blood predicted the clearance of PEGylated liposomal formulations in mice, rats, dogs and patients, including PEGylated liposomal doxorubicin (PLD; Doxil) (Caron et al., 2012). Thus, blood monocytes may play a key role in and be a surrogate marker for NP clearance.

A recent series of investigations have proposed that aggregation of SM drugs, while known to occur *in vitro* and a complicating factor in cell-based assays, has *in vivo* consequences (Seidler et al., 2003; Doak et al., 2010; Owen et al., 2012): it is postulated that "colloid-forming drugs" have different oral absorption and biodistribution than "non-colloid forming drugs". Published literature, however, has not examined whether these phenomena involve the MPS. Thus, we have explored the relationship between colloid-forming tendencies and MPS activity through experimental studies with a series of compounds of known aggregation propensity.

Being able to predict PK parameters in humans based on extrapolated data from animal models is an essential step in the drug development process (Gabizon et al., 2003). The translation of preclinical data in order to predict the safety and efficacy of new compounds in humans is often viewed with skepticism, but it is thought to provide decent correlation when selecting the appropriate PK parameters and mechanism of clearance (Meerum Terwogt et al., 2002; Gelmon et al., 1999). The two most commonly selected preclinical models used in toxicology and toxicokinetic studies of SMs and NPs during drug development are rats and dogs.

One approach to understand the PK characteristics displayed in preclinical models is to use an *ex vivo* phenotypic profiling platform including samples obtained from preclinical models and even humans (van der Bol et al., 2010). A phenotypic profiling platform is an *in vitro* or *ex vivo* test that can be used to quickly assess the biological or biochemical activity of a large number of samples as an indicator of the PK and/or PD of a drug. A MPS profiling platform measuring cellular function in blood could be used to evaluate the relationship between function of the MPS and

Table 1
Summary pharmacokinetic studies performed in mice, rats, dogs, and humans after administration of anthracycline-based NP and SM formulations.

Model: 7–8 week old SCID Mice

Drug Treatment	Dosage	Administration	Animal Count	Time Points Evaluated (after administration)
Doxil	6 mg/kg	IV Bolus ×1	3 ♀	0.083, 1, 6, 24, 48 h
DaunoXome	5 mg/kg	IV Bolus ×1	3 ♀	0.083, 1, 6, 24, 48 h
SP1049C	5 mg/kg	IV Bolus ×1	3 ♀	0.083, 1, 6, 24, 48 h
SM Doxorubicin	3 mg/kg	IV Bolus ×1	3 ♀	0.083, 1, 6, 24, 48 h

Model: 12–18 week old Sprague-Dawley Rats

Drug Treatment	Dosage	Administration	Animal Count	Time points collected
Doxil	1 mg/kg	IV Bolus ×1	3 ♀	0.083, 2, 4, 24, 48 h
DaunoXome	5 mg/kg	IV Infusion (5 min)	3 ♀	0.083, 2, 4, 24, 48 h
SP1049C	5 mg/kg	IV Infusion (5 min)	3 ♀	0.083, 2, 4, 24, 48 h
SM Doxorubicin**	6 mg/kg	IV Bolus ×1	4 ♀	0.083, 4, 24, 48 h

Model: Beagle Dogs

Drug Treatment	Dosage	Administration	Animal Count	Time points collected
Doxil	1.5 mg/kg	IV Infusion (30 min)	3 ♀	EOI, 0.5, 1, 2, 4, 8, 24, 48 h
DaunoXome	1.0 mg/kg	IV Infusion (10 min)	3 ♀	EOI, 0.5, 1, 2, 4, 8, 24, 48 h
SP1049C	1.0 mg/kg	IV Infusion (10 min)	3 ♀	EOI, 0.5, 1, 2, 4, 8, 24, 48 h
SM Doxorubicin**	0.5 mg/kg	IV Bolus ×1	2 ♀	0.083, 0.5, 1, 2, 4, 8, 24, 48 h

Model: Humans

Drug Treatment	Dosage	Administration	Patient Count	Time points collected
Doxil**	40 mg/m^2	IV Infusion (60 min)	15	0.33, 1, 4, 8, 24, 48 h
DaunoXome**	40 mg/m^2	IV Infusion (30–60 min)	4	0.5, 1, 4, 8, 24, 48 h
SP1049C**	70 mg/m^2	IV Infusion (2 mL/min)	7	0.5, 1, 4, 8, 24, 48 h
SM Doxorubicin**	50 mg/m^2	IV Bolus (3 min)	12	0.33, 1, 4, 8, 24 h

Time points for animal PK studies were based on the optimal time point for each drug in each animal model.
EOI: end of infusion.
** Concentration versus time data provided from previously published literature. References are available under Section 'Materials and Methods'.