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Distinct biodistribution of doxorubicin and the altered dispositions mediated by different liposomal formulations

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ABSTRACT

The liposomal formulations of doxorubicin produced distinct efficacy and toxicity profiles compared to doxorubicin solution in cancer patients. This study aims to investigate the drug tissue distribution and the driving force for tissue distribution from doxorubicin solution and two liposomal delivery systems, Doxil and Myocet. These three formulations were intravenously administered to mice at a single dose of 5 mg/kg. Eleven organs, plasma and blood were collected at different time points. Total doxorubicin concentrations in each specimen were measured with LC–MS/MS. Compared to doxorubicin solution, both Doxil and Myocet produced distinct doxorubicin tissue exposure in all 11 tissues. Interestingly, the tissue exposure by Myocet was drastically different from that of Doxil and showed a formulation-dependent pattern. Cmax of doxorubicin in heart tissue by Doxil and Myocet was approximately 60% and 50% respectively of that by doxorubicin solution. The predominant driving force for doxorubicin tissue distribution is liposomal-doxorubicin deposition for Doxil and free drug concentration for doxorubicin solution. For Myocet, the driving force for tissue distribution is predominately liposomal-doxorubicin deposition into tissues within the first 4 h; as the non-PEGylated doxorubicin liposomal decomposes, the driving force for tissue distribution is gradually switched to the released free doxorubicin. Unique tissue distributions are correlated with their toxicity profiles.

1. Introduction

Doxorubicin is a potent chemotherapeutic agent to suppress tumor growth and shows efficacy in different cancer types; however, administration of free doxorubicin solution is not well tolerated in healthy tissues due to its toxicity and is especially known for causing severe cardiomyopathy (Chatterjee et al., 2010; Minotti et al., 2004). Liposomal formulations with slow release of doxorubicin were developed and successfully reduced the cardiac toxicity in cancer treatment (Duncan, 2006; Gabizon and Papahadjopoulos, 1988; Gill et al., 1995). Currently, two liposomal formulations of doxorubicin are commercially available for cancer treatments; one is PEGylated formulation (Doxil, approved by FDA and EU); another one was non-PEGylated formulation (Myocet, approved by EU).

The clinical use of the two forms of liposomal doxorubicin has yielded complex findings: their efficacy and adverse effects (AE) cannot be adequately accounted for by the length of circulation time and level of sustained doxorubicin release in circulation and in tissue extracellular space of liposomal formulations. In a phase II clinical trial with metastatic breast cancer patients, Doxil showed comparable efficacy to the free form of doxorubicin, with significantly reduced cardiotoxicity, myelosuppression, vomiting and alopecia (O'Brien et al., 2004). Doxil also showed prolonged blood circulation time and reduced hepatic clearance (Gabizon et al., 1994). Despite the low risk of cardiotoxicity, Doxil produced new adverse effects: hand-foot syndrome (PPE) and oral mucositis, limiting its use at higher doses (O'Brien et al., 2004; Uziely et al., 1995). On the other hand, clinical trials demonstrated that Myocet also maintained the antitumor efficacy of the free form; in some clinical trials, it showed better efficacy than free form (Batist et al., 2006; Cortes et al., 2009). Myocet has acceptable toxicities, including moderate incidence of cardiotoxic events and

Abbreviations: Cmax, maximum concentration measured; AUC, area under the curve of concentration to time profile.

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the relative absence of PPE compared with Doxil (Batist et al., 2001; Cortes et al., 2009; Lorusso et al., 2007). Interestingly, Hendriks et al. hypothesized that clinically observed differences in tumor inhibition between free doxorubicin *vs*. liposomal doxorubicin may be driven by differences in tumor properties that alter the deposition of the drug into tumor cells, and predicted that some tumors will have properties wherein liposomal delivery system carries the identical amount of drug to its target relative to dosing with free drug (Hendriks et al., 2012).

The clinical results raised several unanswered questions with regard to tissue delivery of different liposomal formulations of doxorubicin. First, are doxorubicin blood (or plasma) concentrations reasonable surrogates of tissue concentrations? Recently, it has been reported that drug plasma level may not indicate the drug tissue level (Müller et al., 2004; Yokoi et al., 2015). The use of plasma concentration to predict the efficacy may mislead the decision and cause poor clinical outcome, especially for those drugs that sufficient accumulation of drug on target tissue is essential (Wolf and Presant, 2004).

Second, are there different tissue distribution profiles between Doxil and Myocet as compared to doxorubicin solution, which are correlated with different safety/efficacy profiles? Doxil's antitumor activity in tumors and doxorubicin distribution in healthy tissues from Doxil delivery have been hypothesized to be caused by the accumulation of free doxorubicin released from the liposomes at or near the tumor site or the normal tissues, and the released drugs are the predominant driving force to enter tumor cells or other cell types in normal tissues for their efficacy and safety profile (Gabizon et al., 2003; Maeda et al., 2000). However, studies showed that nearly 100% of drug detected in patient plasma after Doxil injection was in the liposomal-encapsulated form (Gabizon et al., 1994). The level of free doxorubicin is approximately 1/1000 of the drug encapsulated in liposomes, which suggests Doxil is very stable. In addition, Myocet is made of non-PEGylated liposomes, which has higher doxorubicin leakage than Doxil (Swenson et al., 2003, 2001); therefore, it can be postulated that tissue concentrations of doxorubicin from Myocet may be proportionally higher than those from Doxil due to more doxorubicin leaking out from Myocet. However, no data is available to support this speculation.

The third question, which is even more important, is whether different delivery systems, such as Doxil and Myocet, directly deposit into tissues, so that they will exhibit significant tissue uptake and distribution to alter their pharmacological efficacy in comparison with free doxorubicin. A study using a rodent model by Karathanasis et al. demonstrated that tumor response was directly linked to the liposome's vascular permeability (Karathanasis et al., 2009). Multiscale kinetic modeling by Hendricks et al. also suggested that a high degree of liposome deposition in tumor was critical for the success of liposomal anticancer therapeutics (Hendriks et al., 2012). In addition, Charrois and Allen reported that "empty" liposome (containing no doxorubicin) in combination with free doxorubicin solution administration to mice did not produce its toxicity, the PPE syndrome (Charrois and Allen, 2004).

Finally, what is the driving force, i.e. free doxorubicin release from liposomes or direct liposome tissue deposit for tissue drug distribution in different delivery systems? The answers to these four questions will directly impact the assessment of the bioequivalence of different liposomal formulations of doxorubicin and the drug delivery system in general.

In order to answer the above four questions, in this study, we investigated the blood concentration, tissue distribution, and the driving force for tissue distribution from free doxorubicin and two different liposomal delivery systems. We have assessed the pharmacokinetics and tissue disposition of doxorubicin from solution doxorubicin, PEGylated doxorubicin liposomes (Doxil) and non-PEGylated- liposomes (Myocet) in mice. From tissue distribution, we examined the correlations of tissue doxorubicin exposure with blood doxorubicin concentrations, and compared the driving force for tissue distribution of drug from the three different delivery systems. The data suggested that liposomal drug complexes directly deposited into tissues for drug uptake, which provided different driving forces for drug tissue distribution in the three different delivery systems, and that plasma (blood) drug concentration was not a surrogate for drug tissue distribution of these drug delivery systems.

2. Materials and methods

2.1. Chemicals and reagents

Doxorubicin and daunorubicin powder were purchased from Sigma-Aldrich. Doxorubicin hydrochloride solution and Doxil (doxorubicin hydrochloride liposome from Sun Pharmaceutical Ind, India. FDA considers it equivalent to Doxil) were purchased from University of Michigan Hospital. Myocet (GP-Pharm, Spain) was procured from the EU market courtesy of Celgene Corporation. LC–MS grade acetonitrile was purchased from Sigma-Aldrich. Formic acid (98%, LC–MS grade) was obtained from Fluka. Ultrapure deionized water was supplied by a Milli-Q water system (Millipore, Bedford, MA).

2.2. Animal experiments

All animal experiments were performed in accordance with University of Michigan guidelines covering the humane care and use of animals in research. All animal procedures used in this study were approved by University Committee on Use and Care Animals at the University of Michigan.

Female CD-1® IGS mice (strain code: 022, 6–8 weeks old) were purchased from Charles River Laboratories. Doxorubicin hydrochloride solution, Doxil, or Myocet were administrated to cohorts of mice *via* intravenous (IV) injection at 5 mg/kg. Serial samples of blood, plasma, brain, fat, heart, intestine, kidney, liver, lung, muscle, pancreas, spleen, stomach were collected from predose, 0.08, 0.17, 0.25, 0.5, 0.75, 1, 2, 4, 7, 16, 24, 48 and 72 h post dose. At the given time points, the mice (3 mice/time point) were euthanized and blood samples were immediately collected *via* cardiac puncture using a 25 G needle and 1 mL syringe which were pretreated with Na-Heparin. Plasma samples were collected after the blood samples were centrifuged at a speed of 14,500 rpm for 10 min on a bench-top centrifuge. All other tissue samples were immediately excised from the mouse and rinsed extensively in phosphate-buffered saline (pH 7.4) to remove residual blood and internal contents (only for stomach and small intestine). No perfusion was performed. All the tissues were further ground into powder in liquid nitrogen and stored at −80 °C until further analysis with LC–MS/MS.

2.3. Stock solution, working solution and quality control

Doxorubicin and daunarubicin (internal standard, IS) were individually weighed and dissolved in DMSO to the concentration of 5 mg/mL as stock solutions and stored at −20 °C. Working solutions of doxorubicin were prepared by further diluting the stock solution with acetonitrile at 2–5000 ng/mL. Quality control (QC) working solutions at low, medium and high were prepared using a separately weighed and prepared stock solution. IS solution was diluted in acetonitrile to a final concentration of 500 ng/mL for sample preparation. QC samples were evenly distributed among samples of each batch.