Nanomedicine: Nanotechnology, Biology, and Medicine
xx (2017) xxx–xxx

nanomedicine
Nanotechnology, Biology, and Medicine

nanomedjournal.com

Liposomal encapsulation masks genotoxicity of a chemotherapeutic agent in regulatory toxicology assessments

Jenolyn F. Alexander, BTech, MS, David Aguirre-Villarreal[1], Biana Godin, BPharm, PhD*

Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX

Received 8 July 2016; accepted 22 December 2016

Abstract

The burgeoning application of nanotechnology to a variety of industries including cosmetics, food, medicine and materials has led to the exploration of nanotoxicology as a trending subject of research. However the role of a nanovector, in affecting the mutagenicity of its therapeutic payload has not yet been investigated. In this study, we compare the mutagenicity of the free drug – doxorubicin hydrochloride with its nanoencapsulated form – doxorubicin loaded liposome, using conventional methods required for regulatory approval. Contrary to free doxorubicin, doxorubicin encapsulated liposome expressed a significantly lower mutant frequency in the Ames assay, and was non-genotoxic in the *in vitro* micronucleus assay. Further investigation of the systems' cytotoxicity and their interaction with the bacterial cell envelope, suggests that the modification of the test parameters and release of the encapsulated drug prior to the Ames test show comparable mutagenic potential of the nanotherapeutic system to a free drug.

Key Words: Nano; Liposome; Mutagenicity; Genotoxicity; Toxicity; Ames assay

Nanoparticles are currently used in therapy of various conditions and in cosmetics.[1–3] However, there are limited methods in assessing their genotoxicity. The United States Food and Drug Administration (FDA) has established the National Center for Toxicological Research Nanotechnology and Center for Drug Evaluation and Research Nanotechnology Programs to investigate the toxicity of nanomaterials in FDA regulated products and to establish a standard procedure ensuring the safety of nanotherapeutics.[4,5] Genotoxic studies conducted by FDA and other research groups suggest that common methods used to determine the genotoxicity, when applied to nanoparticles produce inconsistent results lacking conclusive evidence of genotoxic potential.[6–8] Therefore, there is an immediate need to understand the role of nanovectors in ascribing genotoxicity and standardize mechanisms of genotoxic evaluation.

Here, we investigate the genotoxicity of the most frequently used nanotherapeutic, doxorubicin liposomes (Dox-Lip), as compared to its free counterpart, doxorubicin hydrochloride (Dox-HCl). Previous studies using the Ames assay or the bacterial reverse mutation assay have shown Dox-HCl to be mutagenic and clastogenic[9–11] and the empty liposome to be non-mutagenic.[12] However, the mutagenicity and genotoxicity of Dox-Lip as a whole component have not been tested yet.

Methods

The mutagenicity and clastogenicity of Dox-HCl (Sigma Aldrich, USA) and Dox-Lip (Doxoves™, FormuMax Scientific Inc., USA) using histidine dependent strains of *Salmonella typhimurium* – TA98, TA102 and TA1537 (Molecular Toxicology, Inc., USA) in Ames standard plate incorporation method and mammalian Chinese Hamster Ovary (CHO-K1) cells in micronucleus assay, using standard procedures.[13–15] LIVE/DEAD® BacLight™ Bacterial Viability Kit (Molecular Probes, USA) and transmission electron microscopy (TEM) were used to determine if Dox-Lip subdues or masks Dox-HCL mutagenic potential in Ames test.

*Corresponding author at: Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX 77030.
E-mail addresses: bianagodinv@gmail.com, bgodin@houstonmethodist.org (B. Godin).
[1] Present Address: Escuela de Medicina del Tecnológico de Monterrey, Del Carmen, 64,710 Monterrey, Nuevo León, Mexico.

http://dx.doi.org/10.1016/j.nano.2016.12.016
1549-9634/

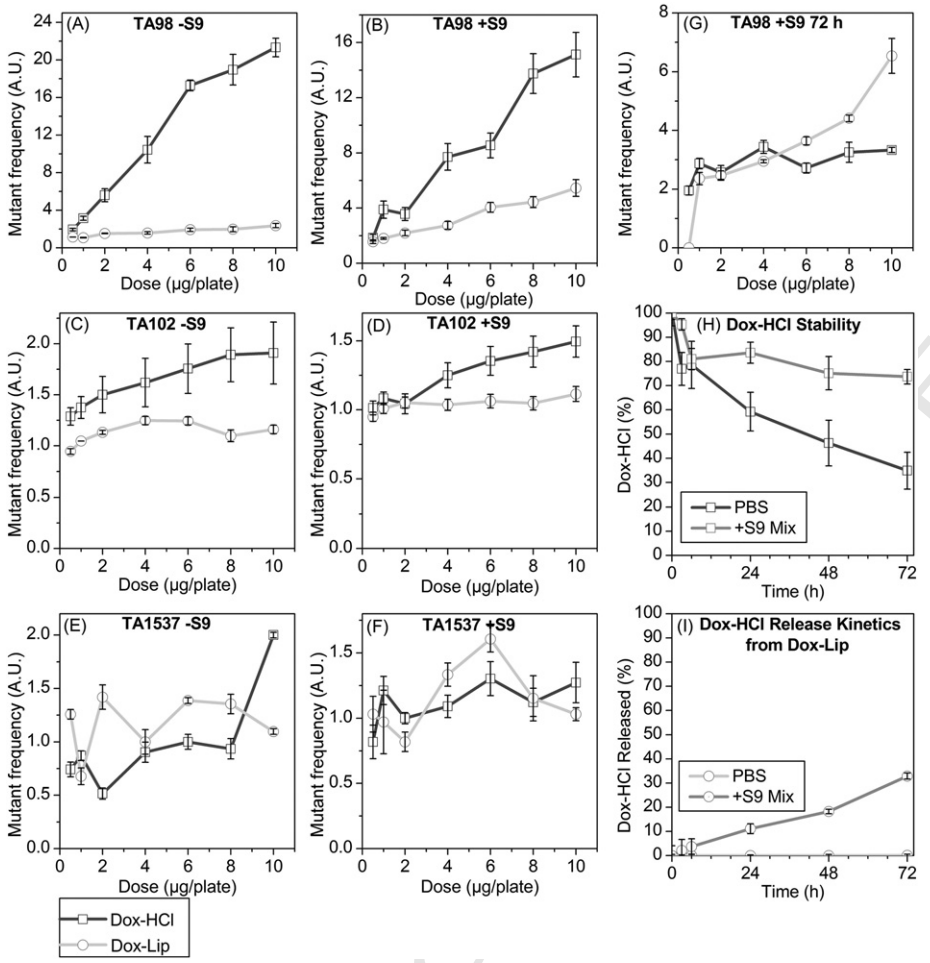

Figure 1. Mutagenicity of Dox-HCl and Dox-Lip by the Ames assay. (**A**-**F**) Mutant frequency of Dox-HCl and Dox-Lip expressed by *Salmonella typhimurium* strains - TA98, TA102 and TA1537, in the absence (−S9) and presence (+S9) of metabolic activation, normalized to spontaneous revertants. (**G**) Mutant frequency expressed by TA98 when Dox-Lip was treated with metabolic enzymes for 72 h prior to the assay. (**H** and **I**) Stability of Dox-HCl and its release kinetics in PBS and S9 mix over time (mean ± SEM, $*p \leq 0.05$).

Results and discussion

The Dox-Lip is comparable to the clinically prescribed Doxil® in physical characteristics with a size range of 80–85 nm, pharmacokinetics with a $t_{1/2}$ of 30 to 40 h, according to the certificate of analysis provided by FormuMax Scientific Inc. At least 4 different batches of Dox-HCl and Dox-Lip were used for the experiments. Since aggregation of liposomes may impact the expression of mutagenicity, Dox-Lip was tested for size-distribution using the Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK). The mean Dox-Lip diameter of various batches was 89.3 ± 0.35 nm (PDI 0.05 ± 0.1) indicating lack of aggregation. The Dox-Lip, as indicated by the manufacturer, has a Dox-HCL concentration of 4 mg/mL (encapsulation efficiency > 99%). Dose-dependent mutant frequency in Ames assay was observed in TA98 and TA102 strains, with Dox-Lip exhibiting significantly lower mutant frequency than Dox-HCl, when the assay was performed with standard methodology,[15] incubating the inoculated plates for 48 h. TA1537 strain showed no dose specific pattern with the same treatment (Figure 1, *A–F*). This behavior is consistent with previous strain comparative studies showing TA1537 to be sensitive only at higher drug doses.[16]

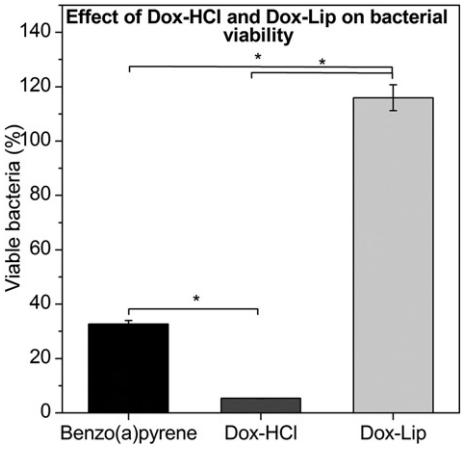

Figure 2. Effect of Dox-HCl and Dox-Lip on the viability of *Salmonella typhimurium* TA98. Benzo(α)pyrene was used as a positive control and data was normalized to treatment control, dilution buffer (mean ± SEM, $*p \leq 0.002$).

Release of drug from nanovectors determines free molecules available for bacterial/mammalian cells at a given time which in turn affects interaction and mutagenicity. The stability of