In Vivo Tumor Toxicity of Doxorubicin Encapsulated in Peptide-Assembled Polylactide Nanoparticles

Xuezhong He, Xiaoming Yang, and Esmaiel Jabbari
Biomimetic and Tissue Engineering Laboratory, Department of Chemical Engineering, University of South Carolina, Columbia, SC 29208, USA
jabbari@engr.sc.edu

Statement of Purpose: Chemotherapy is limited by non-selectivity, causing harmful side effects and reducing the therapeutic efficacy of the drug. Nanopaticles (NPs) provide the opportunity to selectively target the tumor over normal tissue. Our laboratory has developed novel peptide-mediated self-assembled NPs with narrow size distribution, ideal for targeting to the leaky tumor vasculature. The macromer (PLAA-CV6K2) is a conjugate of an acrylate-terminated poly(L-lactide) (PLAA) and a cysteine-terminated $valine_6lysine_2$ (CV6K2) in which the peptide mediates the self-assembly to produce NPs with narrow size distribution while the PLAA provides a hydrophobic matrix for encapsulation of anti-tumor drugs like Doxorubicin (Dox). The objective of this work was to determine circulation time, pharmacokinetics, tumor and host toxicity of Dox-loaded PLAA-CV6K2 NPs.

Methods: Low molecular weight PLAA was synthesized and functionalized with acryloyl chloride as described [1]. The peptide sequence CV6K2 was synthesized as described [2]. Conjugation was performed by coupling the cysteine residue of the peptide sequence to the acrylate group of the macromer. After conjugation, PLAA-CV6K2 (with 2wt% FITC or 5 wt% Dox) was self-assembled by dialysis to form NPs [3]. After self-assembly, the NP suspension was centrifuged to remove the unencapsulated FITC or Dox. PLA NPs stabilized with poly(ethylene oxide) (PLAA-EO) was synthesized [1] as described and used as reference. *In vivo* distribution of the NPs was monitored by near-infrared imaging. The 4T1 breast cancer cells were inoculated under the back skin of syngeneic BALB/c mice. After tail vein injection of the NPs (2 wt% NIR dye), the mouse was scanned tail to head in two infrared channels simultaneously (700 and 800 nm) with the 800 nm channel as reference. For drug pharmacokinetics studies, Dox was extracted from the blood of the animals using the method of Loadman [140]. The residue was re-dissolved in methanol/0.5M-H3PO4 and washed with hexane. The aqueous layer was analyzed by HPLC using a fluorescence detector. For tumor and host toxicity experiments, mice were divided into 6 groups (10 mice/group) when tumors reached 300 mm^3 and received one of these treatments: PBS, PLAA-EO NPs, PLAA-CV6K2 NPs, Dox, Dox+PLAA-EO NPs, and Dox+PLAA-CV6K2 NPs. The injected Dox was 6mg/kg of body weight. After the treatement, body weight and tumor size were measured daily.

Results: PLAA-CV6K2 NPs had 90 nm average size with ±25 nm width but much higher trapping efficiency and longer Dox release compared to CV6K2 NPs (without PLAA conjugation). The NIR dye injected directly in the mouse tail vein peaked after 6 h in circulation and was nearly undetectable after 16 h. On the other hand, FITC loaded in PLAA-CV6K2 NPs peaked after 22 h in circulation and significant amout was present after 30 h in circulation. Pharmacokinetics experiments indicated that Dox coule be detected in the blood above the minimum effective concentration 24 h and 72 h after tail vein injection without and with encapsulation in PLAA-CV6K2 NPs. These results demonstrate that the self-assembled PLAA-CV6K2 NPs prolong the residence time of Dox in the circulation at therapeutic levels. Body weight and tumor size after Dox treatment with and without encapsulation in NPs are shown in Figurs 1a and 1b, respectively. PBS (yellow), PLAA-EO NPs (light green), and PLAA-CV6K2 (pink) groups had weight gain suggesting NPs alone did not have host toxicity. The Dox (blue) group had the highest host toxicity (Fig. 1a). Dox+PLAA-CV6K2 (red) treatment had the same tumor toxicity as Dox (Fig. 1b), while it had significantly lower host toxicity (Fig. 1a). Dox+PLAA-EO NPs had lower host toxicity but tumor toxicity similar to Dox.

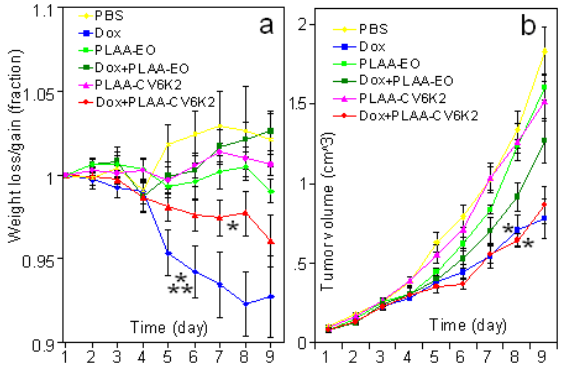

Figure 1: Body weight (a) and tumor volume (b) with time after mice receiving one of the treatments (see text for groups); one star means s.d. (p=0.05) between the test group and PBS, PLAA-EO, PLAA-CV6K2, and Dox+PLAA-EO NPs. Two stars in means s.d. between the test group and Dox+PLAA-CV6K2 NPs.

Conclusion: The results suggest that the interaction of PLAA-CV6K2 NPs with the surface of tumor cells results in higher tumor toxicity (lower tumor growth rate) with significantly reduced host toxicity.

References

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