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$sPLA_2$ sensitive fluid phase liposomes induce severe toxicity in murine cancer model

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Summary: Tuning liposomes for secretory phospholipase A_2 induced release of oxaliplatin results in enhanced anti-neoplastic effects *in vitro* and extensive toxicity *in vivo*.

Introduction

The clinical use of liposomal drug delivery vehicles is often hindered by insufficient drug release[1]. One compelling solution is utilizing an endogenous trigger mechanism that relies on a difference in the microenvironment of healthy and diseased tissue, such as the elevated expression of endogenous enzymes in cancerous tissues.

Secretory phospholipase A_2 ($sPLA_2$) is an interfacially active lipase which functions mainly on organized substrates, such as lipid bilayers[2], and it seems to have a preference for structural defects, such as domain boundaries[3,4]. We hypothesized that by adjusting the level of cholesterol in anionic, unsaturated liposomes we could tune the surface defects[5,6], and as such tune the enzyme sensitivity, thus obtaining liposomes with an improved therapeutic outcome and reduced side effects.

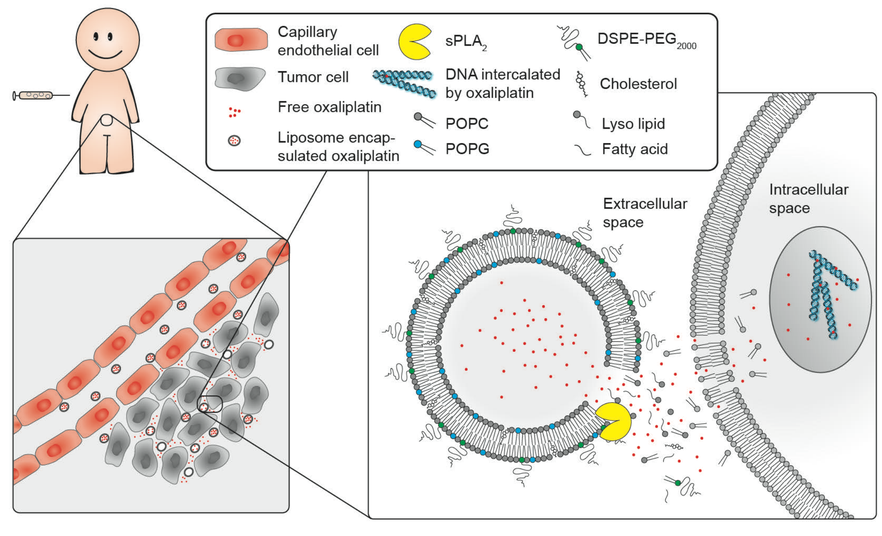
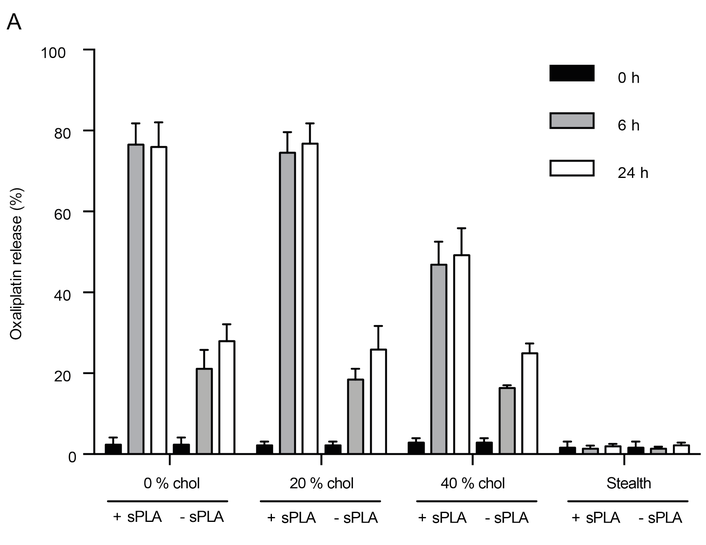


Figure 1: Conceptual illustration. Liposome encapsulated oxaliplatin will circulate until it encounters the fenestrated capillaries in the tumor tissue, where it extravasates. Here it encounters an elevated level of secretory phospholipase A_2 ($sPLA_2$), which hydrolyses the phosphoglycerolipids, causing release of the drug. In addition the hydrolysis products, lyso-lipids and free fatty acids, may act as permeability enhancers, thus further contributing to drug transport across the cellular membrane.

Results

Liposomal release of oxaliplatin is $sPLA_2$ dependent, but only slightly cholesterol dependent



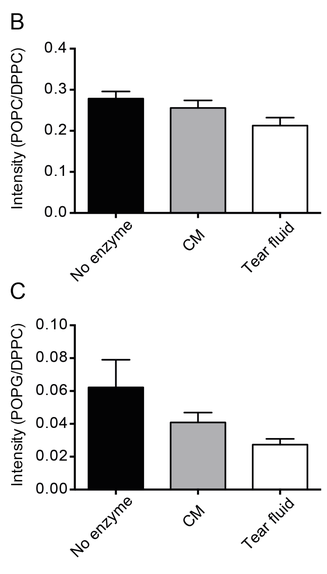


Figure 2: Enzymatic hydrolysis and release of oxaliplatin. **A)** POPC/POPG/Chol/DSPE-PEG2k liposomes with 0, 20 and 40 % cholesterol and HSPC/Chol/DSPE-PEG2k (Stealth) liposomes, loaded with oxaliplatin, were incubated in the presence or absence of secretory phospholipase A_2 (± $sPLA_2$) at 37 °C with gentle magnetic stirring for 0, 6 and 24 h. Release of oxaliplatin was determined by ICP-MS. Values are mean of three individual experiments ± SD. POPC/POPG/Chol/DSPE-PEG2k (35:40:20:5) liposomes were incubated with colo205 cell conditioned media (CM), human tear fluid or no enzyme at 37 °C with gentle magnetic stirring for 6 h. Hydrolysis of **B)** POPC and **C)** POPG was determined by MALDI-TOF MS. DPPC was included as internal reference. Values are mean of a minimum of three lipid extractions and three spots of each extraction.

Liposomes show enhanced cytotoxic effect *in vitro*

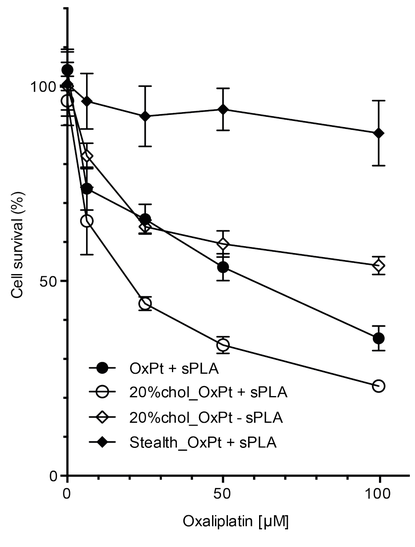


Figure 3 *In vitro* cytotoxicity of oxaliplatin (OxPt) loaded $sPLA_2$-sensitive liposomes. HT-29 cells were treated with an increasing amount of OxPt loaded POPC/POPG/Chol/DSPE-PEG2k liposomes with 20 % cholesterol, either in the presence (open circles) or absence (open diamonds) of colo205 cell conditioned media containing $sPLA_2$. OxPt in free form (closed circles) or encapsulated in HSPC/Chol/DSPE-PEG2k (Stealth) liposomes (closed diamonds) were used as controls. Cell survival was evaluated by MTS staining. Values are mean of triplicates with blank (media with MTS reagent) subtracted ± SD. All values are normalized to non-treated cells. The data is representative of minimum three separate experiments.

$sPLA_2$ responsive liposomes induce severe toxicity *in vivo*

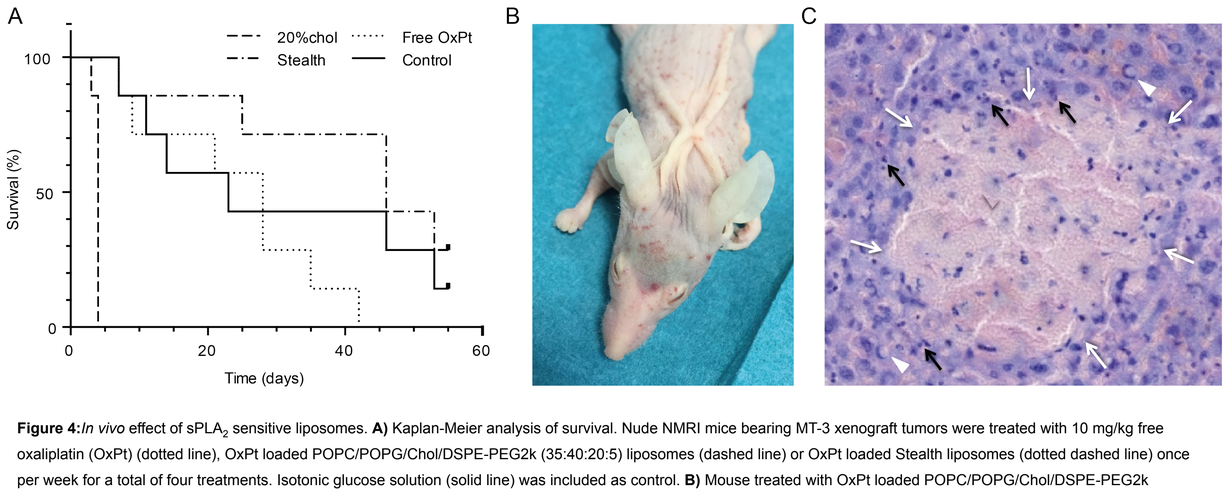


Figure 4:*In vivo* effect of $sPLA_2$ sensitive liposomes. **A)** Kaplan-Meier analysis of survival. Nude NMRI mice bearing MT-3 xenograft tumors were treated with 10 mg/kg free oxaliplatin (OxPt) (dotted line), OxPt loaded POPC/POPG/Chol/DSPE-PEG2k (35:40:20:5) liposomes (dashed line) or OxPt loaded Stealth liposomes (dotted dashed line) once per week for a total of four treatments. Isotonic glucose solution (solid line) was included as control. **B)** Mouse treated with OxPt loaded POPC/POPG/Chol/DSPE-PEG2k (35:40:20:5) liposomes, euthanized three days post first treatment due to excessive weight loss, dehydration and subcutaneous bleedings. **C)** Liver section from a mouse treated with OxPt loaded POPC/POPG/Chol/DSPE-PEG2k (35:40:20:5) liposomes. The liver section displays extensive multifocal necrosis of hepatocytes (white arrows) with collapse of hepatic sinusoids and hydropical injury to the cell nucleus of the hepatocytes. Several of the hepatocytes display nuclear inclusion bodies and vacuolar degeneration (white arrowheads). Extensive inflammatory reaction is seen in the hepatic sinusoids and around necrotic regions with accumulation of granulocytes (black arrows) (HE stained FFPE 5 um). .

Conclusion

Tuning the amount of cholesterol did not seem to considerably alter the enzyme sensitivity towards the novel $sPLA_2$ sensitive liposomes (SSLs). Oxaliplatin loaded SSLs revealed efficient *in vitro* growth inhibition compared to clinically used stealth liposomes. However, treatment of nude NMRI mice induced severe toxicity, demonstrating that great caution should be implemented when using $sPLA_2$ sensitive liposomes, and that the real utility can only be revealed *in vivo.*

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