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sPLA$_2$ sensitive fluid phase liposomes induce severe toxicity in murine cancer model

Ragnhild G. Østrem$^a$, Ole L. Nielsen$^b$, Anders E. Hansen$^a$, Thomas L. Andresen$^a$

$^a$Department of Molecular and Nanotechnology, Technical University of Denmark, Department of Micro- and Nanotechnology, Center for Nanomachinery and Theranostics, Technical University of Denmark, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3, 1870 Frederiksberg, C, Denmark. $^b$Department of Molecular Imaging, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen N, Denmark.

**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. 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, and it seems to have a preference for structural defects, such as domain boundaries. We hypothesized that by adjusting the level of cholesterol in anionic, unsaturated liposomes we could tune the surface defects, and as such tune the enzyme sensitivity, thus obtaining liposomes with an improved therapeutic outcome and reduced side effects.

**Results**

Liposomal release of oxaliplatin is sPLA$_2$ dependent, but only slightly cholesterol dependent

Liposomes show enhanced cytotoxic effect *in vitro*

Figure 1: Conceptual illustration. Liposome encapsulated oxaliplatin will circulate until it encounters fenestrated capillaries in the tumor tissue, where it extravasates. Here it encounters an elevated level of secretory phospholipase A$_2$, sPLA$_2$, which hydrolyzes the phospholipids, releasing oxaliplatin. In addition the hydrolysis products, free fatty acids, may act as permeability enhancers, thus further contributing to drug transport across the cellular membranes.

**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*.

**References**


**Contact information:**

Ragnhild Garborg Østrem
Technical University of Denmark
ragga@nanotech.dtu.dk

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**Figure 2:** Biophysical hydrolysis and release of oxaliplatin. A) POPC/POPG/cholesterol/DPPE-PE/PP19-59{sPLA$_2$} liposomes with 5, 10 and 15 mol% cholesterol, loaded with oxaliplatin, were incubated in the presence of or absence of secretory phospholipase A$_2$. B) POPC/POPG/cholesterol/SPHPE-PE (Stealth) liposomes, loaded with oxaliplatin, were incubated in the presence or absence of secretory phospholipase A$_2$. C) Numerical data (mean ± SD) of the cytotoxicity hypothesis. Values are mean of triplicate experiments and three replicates of each condition.

**Figure 3:** Liposomes show enhanced cytotoxic effect *in vitro*. A) Time course of cytotoxicity of oxaliplatin loaded POPC/POPG/cholesterol/DPPE-PE (open squares) or POPC/POPG/cholesterol/SPHPE-PE (Stealth) liposomes (closed diamonds) once per week for a total of four treatments. Isotonic glucose solution (solid line) was included as control. B) Representative MTS staining of colo205 cell conditioned media containing POPC/POPG/cholesterol/DPPE-PE (open squares) or POPC/POPG/cholesterol/SPHPE-PE (Stealth) liposomes (closed diamonds) once per week for a total of four treatments. Isotonic glucose solution (solid line) was included as control. C) Representative MTS staining of colo205 cell conditioned media containing POPC/POPG/cholesterol/DPPE-PE (open squares) or POPC/POPG/cholesterol/SPHPE-PE (Stealth) liposomes (closed diamonds) once per week for a total of four treatments. Isotonic glucose solution (solid line) was included as control.