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Redox-Sensitive Liposomes for Glioblastoma Treatment

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Introduction

Treatment of glioblastoma remains a challenge due to inability of the drug to reach the intracellular target. Invasive glioblastoma is associated with high grade vascularization and break-down of the blood-brain barrier (BBB), which could aid in delivering drugs to the tumor site. However, once at the tumor site, the drug has to be internalized and transported to the specific target.

The aim of the current project is to develop a drug delivery system (DDS) that crosses the permeable BBB to specifically target invasive glioblastoma cells and thereby facilitate uptake. Furthermore the DDS will be activated in the tumor environment to escape the endosome and drug efflux mechanisms, thereby transporting the drug to the intracellular target. The DDS consists of a positively charged liposome formulation and redox-sensitive lipopeptides (RSL) or non-cleavable lipopeptides (nCL) with a PEG-linker that shield the positive charge. For intracellular cleavage a cell-penetrating (CP) moiety (8-arginines or 8R) is furthermore included. The chemotherapeutic drug Doxorubicin (DOX) is loaded into the DDS for cytotoxicity experiments. The DDS concept and components are shown in Figure 1.

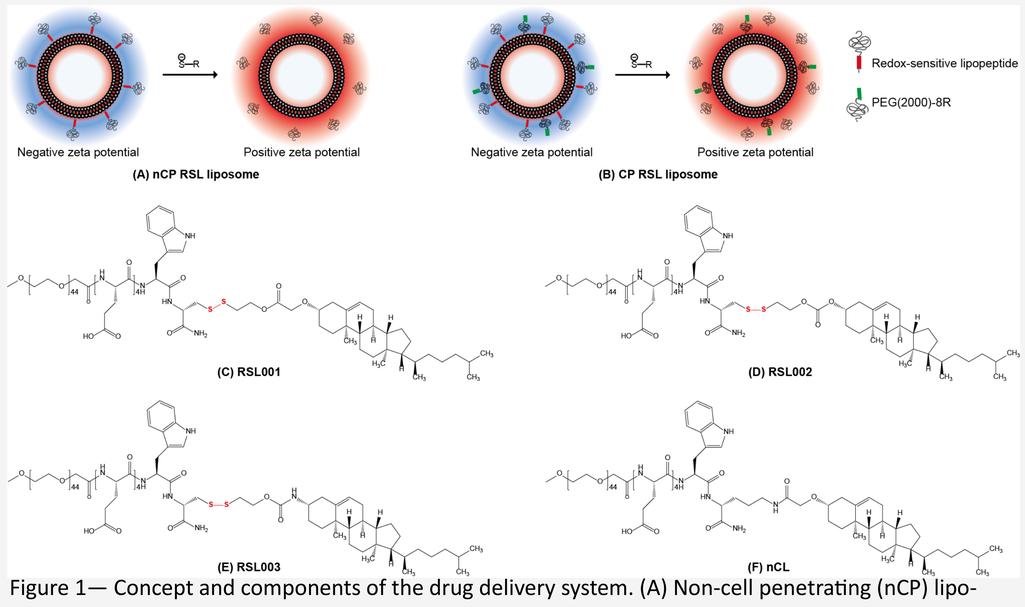
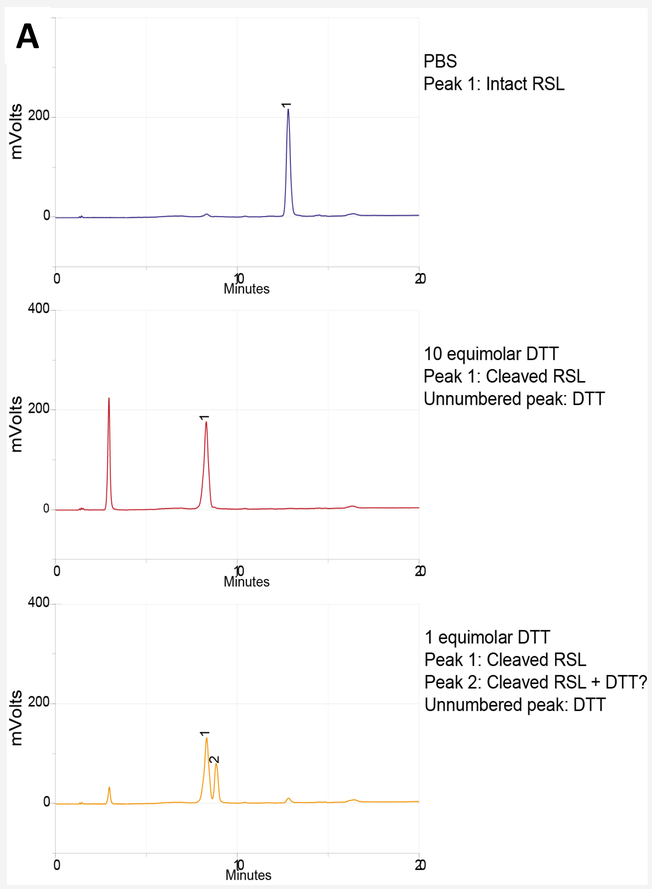
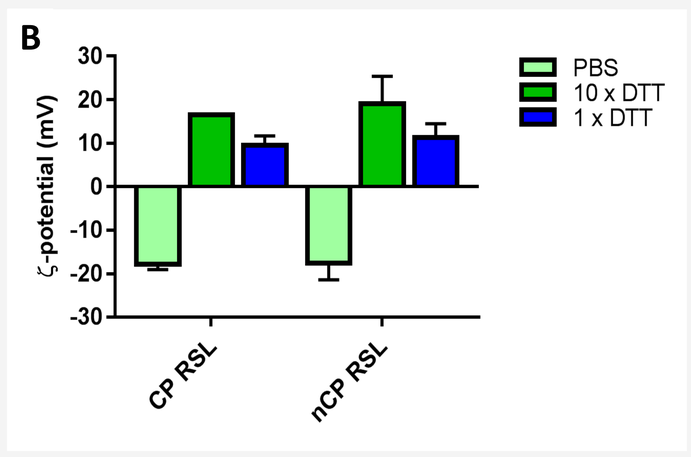
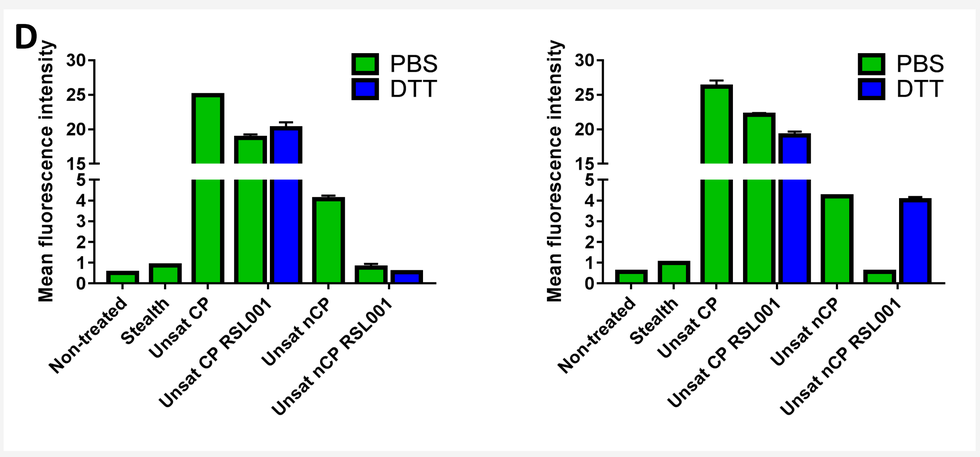


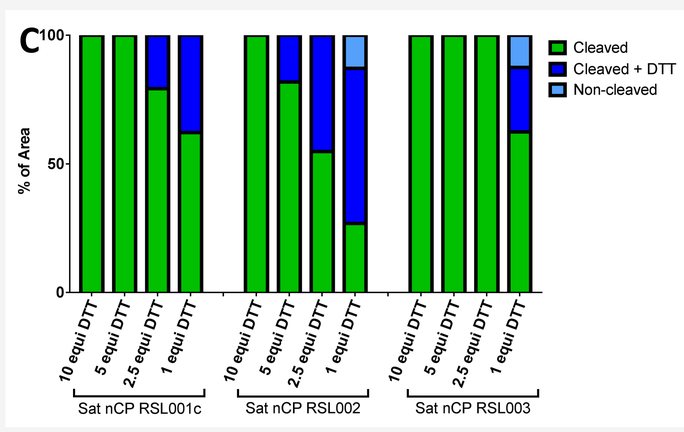
Figure 1— Concept and components of the drug delivery system. (A) Non-cell penetrating (nCP) liposomes, (B) Cell penetrating (CP) liposomes, (C) Esther s-s construct, (D) Carbonate s-s construct, (E) Carbamate s-s construct, (F) Lysine construct

Results









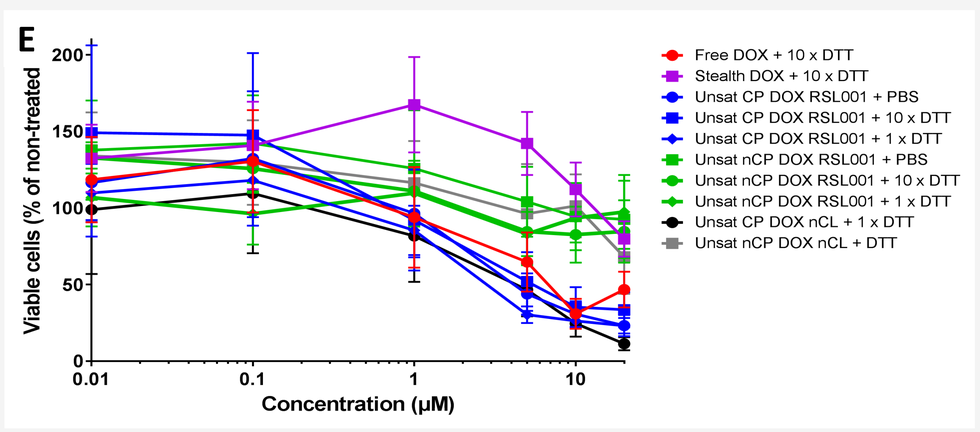


Figure 2—Cleavage, charge-reversal, uptake, and cytotoxicity results. (A) HPLC chromatograms of intact and cleaved RSLs in liposomes, (B) Charge of RSL liposomes with PBS or DTT treatment, (C) HPLC analysis of cleavage of RSLs in saturated liposomes (5 % DOTAP), (D) Uptake in U87 cells of unsaturated RSL liposomes (7.5 % DOTAP) with PBS or DTT treatment, (E) Cytotoxicity to U87 cells of RSL and nCL liposomes (5 % DOTAP) with PBS or DTT treatment

Cleavage and Charge-reversal

HPLC analysis (Figure 2A) showed that the intact lipopeptide eluted after 13 minutes. Treatment with 10 equimolar DTT to RSL resulted in 100 % of the RSLs being cleaved (one peak at 8 minutes), while treatment with 1 equimolar DTT resulted in the fully cleaved peak and an extra peak, which was believed to be the cleaved RSL with DTT still attached.

Charge reversal was proven by zeta-potential measurements of the RSL liposomes prior to and after treatment with DTT (Figure 2B).

A cleavage experiment (Figure 2C) indicated that the cleavage kinetics of RSL001, RSL002, and RSL003 was different with RSL001 and RSL003 being cleaved faster than RSL002 and RSL003 showing less tendency to create the DTT intermediate.

Uptake and Cytotoxicity

Uptake in U87 cells (Figure 2D Left) showed that CP liposomes had high uptake compared to stealth regardless of treatment (PBS or DTT). Thus, the RSLs did not shield the uptake effect of the 8Rs and these liposomes could therefore be used to assess the effect of intracellular cleavage. For the nCP liposomes it was shown that the uptake was 9 fold higher than stealth prior to post-insertion of RSL001 and that the uptake was completely inhibited by post-insertion of RSL001. Treatment with DTT could reverse the effect of the post-insertion and returned the uptake to the same level as pre-post-insertion. An issue that did arise was the inability to cleave the RSLs in the unsaturated liposomes, leading to incapability of restoring the uptake pre-post-insertion (Figure 2D Right). The same issue arose in the cytotoxicity experiment (Figure 2E), where all the CP RSL and nCL liposomes showed high toxicity, while the nCP (even the DTT treated) was toxic comparable to stealth.

Conclusion and Perspectives

It has been shown that RSLs can be successfully post-inserted into liposomes, thereby changing the charge and the uptake properties of the liposomes. Furthermore, cleavage of the RSLs can restore the initial properties of the liposomes. Cleavage of the three RSLs indicated different cleavage kinetics and more investigations into these kinetics and the impact on uptake will be undertaken. In the unsaturated formulation the RSLs were not cleavable after storage and the stability of the RSLs in unsaturated and saturated formulations will therefore be investigated.

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Research Initiative on Brain Barriers and Drug Delivery