Low Temperature Sensitive Liposome Mediated Delivery of Thrombolytic Agents

Vishal Saxena\textsuperscript{1,2}, Carmen Gacchina\textsuperscript{1,3}, Ayele Negussie\textsuperscript{1}, Pavel Yarmolenko\textsuperscript{1,4}, Ari Partanen\textsuperscript{1,5}, Karun Sharma\textsuperscript{1,6}, Bradford Wood\textsuperscript{1}, Matthew Dreher\textsuperscript{1}

\textsuperscript{1}Center for Interventional Oncology, Radiology & Imaging Sciences, Clinical Center, National Institutes of Health, Bethesda, MD, USA, \textsuperscript{2}Howard Hughes Medical Institute - National Institutes of Health Research Scholars Program, Bethesda, MD, USA, \textsuperscript{3}Imaging Science Training Program, National Institutes of Health, Bethesda, MD, USA, \textsuperscript{4}Duke University, Department of Biomedical Engineering, Durham, North Carolina, USA, \textsuperscript{5}Philips Healthcare, Cleveland, OH, USA, \textsuperscript{6}Department of Radiology, Georgetown University, Washington D.C, USA

Introduction: Thromboembolic disease (e.g. myocardial infarction, stroke, deep vein thrombosis) causes significant morbidity and mortality worldwide. Thrombolytics, a mainstay of therapy, have been shown to effectively dissolve thrombi and recanalize occluded blood vessels. However, their effectiveness is limited clinically by lack of specific delivery, which necessitates large therapeutic doses, resulting in systemic toxicity. Temperature-sensitive liposomes preferentially release their contents upon reaching mild hyperthermia. Together with a hyperthermia applicator, such liposomes have the potential for local, sustained delivery of thrombolytic agents at the site of a heated thrombus. The objectives of this study were 1) to formulate liposomes encapsulating thrombolytics, 2) to characterize release, and 3) to ensure thrombolytic activity following hyperthermia.

Methods: Three liposome formulations were investigated: temperature-sensitive liposome (TSL; DPPC:DSPE-PEG2000 (95:5)), low temperature-sensitive liposome (LTSL; DPPC:MSPC:DSPE-PEG2000 (85.3:9.7:5.0)), and traditional temperature-sensitive liposome (TTSL; DPPC:HSPE:Chol:DSPE-PEG2000 (55:25:15:5)). The enzymatic thrombolytics staphylokinase (SAK), urokinase (UK), or tissue plasminogen activator (tPA) were loaded into each liposome formulation. To characterize time and temperature dependence of a high MW cargo from each formulation, fluorescein-conjugated dextrans (70kDa) were loaded (25mg/ml) and release was quantified with spectrophotometric methods. Thrombolytic leakage (37°C) and activated release with heating at the temperatures that exhibited maximal release rate (38-44°C) were quantified via chromogenic enzymatic activity assays to determine the thrombolytic-liposome formulation with the highest release/leakage rate ratio. Clot lysis was evaluated by measuring mass of whole blood clots.

Results & Conclusion: The LTSL formulation was found to have the optimal release characteristics with heat activation (max. release temperature of 41.3°C), and the SAK LTSL yielded significant advantages over other thrombolytic-liposome pairs in terms of leakage to release ratio. Release of dextrans loaded in LTSLs was observed to be 11.5±1.5%, 79.7±1.6%, and 94±4% after 15 min in plasma at 37°C, 39°C, and 41.3°C, respectively. SAK LTSL had the highest release/leakage ratio of 8.3±0.6. SAK LTSL also demonstrated the greatest whole blood clot lysis with hyperthermia (84±8% and 45±8%, at 42°C and 37°C, respectively), p<0.05; n≥3 for all cases. The SAK LTSL is a temperature-sensitive thrombolytic-liposome formulation that can potentially deliver thrombolytics selectively when combined with local hyperthermia, thus providing sufficient thrombolysis while minimizing systemic side effects. Despite reduced enzymatic activity for SAK at temperatures above 37°C, this approach remains significant and achieves clot lysis in vitro. Future studies will combine this liposome formulation with magnetic resonance guided high intensity focused ultrasound for image-guided focal heating and clot lysis.