102e Targeted Delivery of Thrombolytic Agents Using Complexation and Conjugation with Dendritic Polymers

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Streptokinase is used for treatment of deep vein thrombosis and myocardial infarction in a critical care setting. Current therapy is suboptimal due to the short half-life of streptokinase and inactivation of streptokinase by circulating antibodies. Dendrimers are promising nanovehicles for delivering drugs and diagnostic agents to the target site. PAMAM-G3.5-COOH and NH2 terminated dendrimers were used in the present research to complex/conjugate streptokinase and thus improve the half-life and reduce its immunogenicity to achieve improved delivery of streptokinase to the thrombus. We hypothesize that enhanced delivery of SK will occur because dendrimers (~ 20 nm) may be able to diffuse better into the clots than particulate delivery systems. This will be enhanced when targeting antibodies are attached. These drug delivery systems can protect the protein from antigens by encapsulation or by blocking its antigen binding amine groups while their micro/nanostructures can target the clots. To compare the performance of the dendritic delivery systems with traditional methods, the drug was encapsulated in PLGA microparticles also. The objective of this work is the preparation, in vitro and in vivo characterization of dendritic nanodevices for clot lysis.

Streptokinase was complexed to the COO-Na+ groups of PAMAM-G3.5 COOH dendrimer by protonating the amine groups of streptokinase. On the other hand, the conjugate was formed by covalently linking the amine groups of the protein to the -COOH groups of PAMAM-G3.5-dendrimer. Streptokinase is also encapsulated in PLGA microparticles by using double emulsion solvent evaporation method. The protein content in the formulations has been estimated using Lowry's protein estimation method and its activity has been checked using chromogenic assay. The activity of streptokinase was assessed using a chromogenic assay. The chromogenic assay showed that both complexed, and conjugated streptokinase were biologically active. From kinetic analysis it was observed that complexed and conjugated streptokinase took longer time than free streptokinase to hydrolyze the peptide substrate. Further studies are underway to test the superiority of the dendritic nanodevices in vivo using a rabbit thrombus model using time and extent of reperfusion and decrease in clot weight as therapeutic end points. The reperfusion time in is measured by monitoring the blood flow in jugular vein using Doppler flowmetry. Increased activity of streptokinase has been observed due to increased half-life and clot targeting. Current work focuses on the use of antibodies to target the clots. Resulted will be presented for the in vivo performance of the dendrimer formulated streptokinase and the PLGA system.