Nitric Oxide-Releasing Dendrimers for Targeted NO Therapy

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Statement of Purpose: Local drug delivery and cell-targeted drug delivery are the increasingly popular alternatives to systemic therapies, which are often unable to distribute beneficial doses efficiently and without toxic side effects. However, exposure of the tissue to the drug is fleeting in these systems, and targeting therapeutic agents to specific cells at sites of localized pathologies is currently under investigation as an optimized method for local drug delivery, increasing specificity as well as retention of the drug at the disease site. The work presented here introduces diazeniumdiolate-modified lysine dendrimers for targeted, sustained delivery of nitric oxide (NO) to locations of active vascular disease.

Methods:

Synthesis and characterization of PEG-core polylysine dendrimers

Branched lysine dendrons were synthesized by standard fluorenylmethoxycarbonyl (Fmoc) chemistry on a peptide synthesizer using N_α-N_ε-di-Fmoc-L-lysine. Briefly, a 4-molar equivalents of di-Fmoc-lysine was bound to Fmoc-Lysine(Boc) resin deprotected, then reacted with another 4-fold excess of di-Fmoc-lysine, iteratively building a branching structure. The procedure was repeated a total of 4 times to build generation 4 dendrons. Dendrimers were synthesized after reacting dendrons with NO gas in water to protect the terminal amines from reaction, then mixing 4 molar equivalents of lysine dendrons with a 4-arm PEG-amine for 4 hours in anhydrous DMF, precipitated in ethyl ether and filtered. Fluorescent dendrimers were synthesized by adding fluorescein isothyocyanate (FITC) to dendrons prior to reaction with PEG-amine. Targeted dendrimers were formulated using an avidin-biotin linkage. Dendrons were biotinylated through reaction with biotin-NHS in anhydrous DMF and later incubated with SLe^X-biotin that was previously reacted with 2 molar equivalents of avidin in HBS. Fluorescent, targeted, NO-releasing dendrimers could be synthesized with the addition of previously functionalized dendrons to PEG-amine.

NO release

PEG-Lys dendrimers were reacted with NO gas in water under argon at room temperature overnight. A Ninhydrin assay was used to quantify the number of amines present before and after the reaction with NO. Dendrimers were then incubated at 37°C in HBS, and NO release was measured using the Griess assay, which quantifies nitrites, the primary degradation product of NO.

Platelet adhesion

NO-releasing dendrimers were dissolved in deionized water and sterile filtered. Collagen I was adsorbed onto glass coverslips to provide a thrombogenic reference material. Heparin and mepacrine, which fluorescently labels platelets, were both added to whole blood obtained from a healthy volunteer. Blood was exposed to NO-releasing dendrimers for 20 minutes at 37°C. Collagen I films were incubated with the mepacrine-labeled blood at 37°C for 20 minutes to allow binding of platelets, and adhesion was quantified by fluorescence microscopy.

Targeting studies

Fluorescent, targeted dendrimers were dissolved in deionized water and sterile filtered. HUVECs were seeded in tissue culture wells and allowed to adhere for 24 hours, then stimulated with IL-1β for 4 hours to encourage the presentation of cell adhesion molecules.Cells were then exposed to either fluorescent SLe^X-conjugated dendrimers or to fluorescent dendrimers that had no SLe^X for 30 minutes. All media was aspirated and the cells all rinsed thoroughly three times with sterile PBS. Dendrimers bound to the surface of the HUVECs were visualized using a fluorescent microscope.

Results / Discussion:

NO Release

NO release from PEG-Lysine dendrimers occurred for over 60 days under physiological conditions (Figure 1).

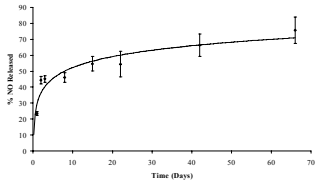

Figure 1. NO release from PEG-Lysine-NO dendrimers.

Platelet Adhesion

Platelet adhesion in the presence of NO-releasing dendrimers was drastically reduced (Figure 2).

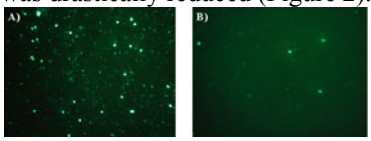

Figure 2. Platelet adhesion of whole blood exposed to A) Control dendrimers and B) NO-releasing dendrimers.

Targeting Studies

Targeted dendrimers containing SLeX preferentially bound the surface of HUVECs stimulated by IL-1β (Figure 3). Dendrimers that did not have SLeX did not bind cells.

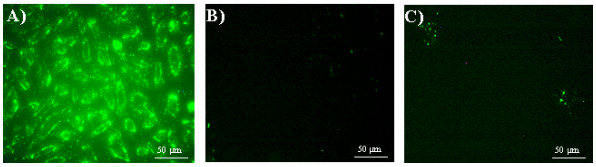

Figure 3. A) Targeted dendrimers bound stimulated HUVECs, B) Non-targeted dendrimers did not bind, and C) targeted dendrimers did not bind non-stimulated cells.

Conclusions:

These bioactive polymers may have applications in further analysis of the effects of NO in biological systems and may prove beneficial as drug delivery systems in numerous applications, offering the ability to design injectable, targeted therapeutics.