Targeting Efficiency Optimization of RGD-Modified Liposomes to Activated Platelets

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Introduction: Site-specific delivery could be used to aid in the management of thrombosis and re-occlusion following cardiology procedures such as balloon angioplasty and intracoronary stent placement. In previous studies, we demonstrated the feasibility of employing a linear Arg-Gly-Asp (RGD) containing peptide as the motif for targeted delivery of liposomes to activated platelets[1]. Here we attempt to improve the targeting ability of liposomes to activated platelets by modulating the affinity of the RGD peptide ligand.

Methods: Amino acid derivatives, activator (1-hydroxy-1-azabenzotriazoleuronium, HATU), and synthesis resin were purchased from Anaspec, Inc. (San Jose, CA). An activated, purified synthetic polyethylene oxide (PEO) derivative of distearoylphosphatidylethanolamine (DSPE-PEO) containing a terminal N-hydroxysuccinimide (NHS) activated carboxyester was purchased from NOF America Corporation. Linear RGE (l-RGE), linear RGD (l-RGD) and cyclic RGD (c-RGD) peptides were synthesized by solid phase peptide synthesizer. The cyclic RGD conformation was obtained by oxidizing the two terminal cysteins to form a disulfide. Peptide binding activity was studied with a competitive assay using an aggregometer. The binding of liposomes to activated platelets adsorbed on coverslips and platelets in suspension was characterized by fluorescence microscopy and flow cytometry respectively, and the effect of liposome binding on platelet activation and aggregation was investigated by aggregometry.

Results / Discussion: Binding of fluorescence labeled liposomes to platelets adsorbed on coverslips was studied by fluorescence microscopy. Fibrinogen was introduced to distinguish differences in binding affinity. Results shown in Figure 1 suggest only the binding affinity of c-RGD-liposomes is strong enough to compete with fibrinogen in binding to activated platelets.

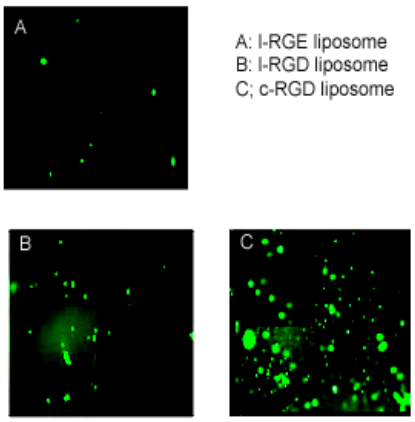

Figure 1 Fluorescence micrographs for platelets adhered onto collagen III coated glass coverslips and incubated with test liposomes bearing different peptide motifs in the presence of fibrinogen. The results shows that c-RGD-liposomes are able to compete with fibrinogen in binding to adsorbed platelets.

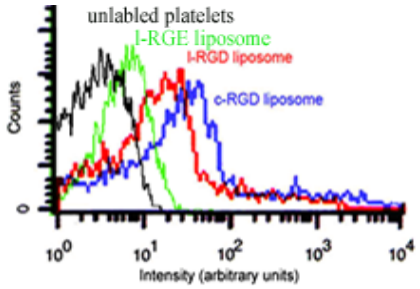

Figure 2 Flow cytometry results from analysis of activated platelets incubated with test liposomes show the progressive increase of binding affinity from l-RGE- to l-RGD- to c-RGD-liposomes.

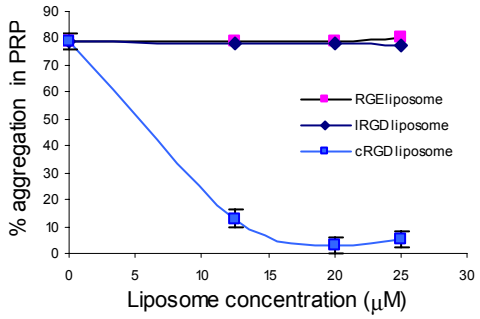

Figure 3 Aggregometry analysis of platelet aggregation in the presence of test liposomes at different concentrations shows only c-RGD-liposomes are able to inhibit platelet aggregation significantly.

Activated platelets in suspension were also incubated with test fluorescence labeled liposomes, and fluorescence intensity of platelets was examined by flow cytometry. Results shown in Figure 2 indicate the c-RGD-liposomes have higher binding affinity than l-RGD-liposomes. The binding affinity of negative control l-RGE-liposomes is negligible.

The aggregometry analysis shows that c-RGD-liposomes significantly inhibit platelet aggregation, probably due to their high binding affinity which enables the effectual competition with fibrinogen in binding to activated platelets. In contrast, l-RGD-liposome did not affect platelet aggregation at examined concentrations.

Conclusions: The binding ability and targeting efficiency of liposomes can be optimized by designing targeting motif with appropriate binding affinity. Binding affinity of RGD-liposomes was significantly improved by surface modification with cyclic RGD peptide.

References:

1 Sen Gupta A, Huang G, Lestini B, Sagnella S, Kottke-Marchant K, Marchant RE. Thromb Haemost. 2005; 93:106-114.