P-047 **Target-sensitive liposomes for the delivery of thrombolytic agents**

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INTRODUCTION AND OBJECTIVES

Vascular thrombosis is a major clinical problem, particularly in developed Western countries. The best way to improve patient survival and decrease morbidity is prompt detection and treatment of thrombosis with thrombolytic agent(s). A variety of thrombolytic agents such as streptokinase (SK), urokinase and tissue plasminogen activator (t-PA) are pharmacological active and available. However, the systemic side effects of these agents (systemic fibrinogenolysis and bleeding) raise and impose unavoidable clinical difficulties. These side effects are the result, at least in part, of the fact that these therapeutic agents comprise proteolytic components of the blood clotting cascade, and thus their thrombolytic capabilities are also responsible for their capacity to disrupt hemostasis systemically, particularly at dosages which are required and used for therapeutically effective effects of drugs at the thrombus site. Thus, the outcome of clinical administration of these agents would obviously be improved if a thrombolytic agent could specifically delivered targeted to the thrombus sites *in vivo*, thereby reducing the incidence of unwanted systemic side effects (Vyas and Vaidya, 2009).

The main objective of the present study is to increase the accumulation of SK in the thrombus and to increase the clot lysis activity of the SK. In the present investigation, target-sensitive (TS) liposomes were prepared which not only target the thrombus but also instantly release drug following the interaction with the activated platelets embedded in the thrombus by self-destruction mechanism. Thus higher concentration at the target site may be achieved with reduced side effects.

MATERIALS AMD METHODS

Synthesis of acylated peptide

Synthesis of dipalmityl-peptide was performed in two steps. di-BOC lysine was firstly conjugated to the free amine group of the c(RGDfK) by carbodiimide chemistry. The peptide having an amino-terminal lysine with two free amino groups was conjugated to NHS-palmitate synthesized by procedure reported earlier (Shen et al., 2007).To confirm the activity or binding ability of modified peptide, platelet aggregation study was performed according to the procedure reported earlier by Huang et al., 2008, with modification. Half maximal inhibitory concentration (IC_{50}) value for the peptide and modified peptide was calculated.

Development of target sensitive liposomes

TS liposomes were prepared using DOPE as a constituting lipid by the procedure reported earlier by Ho et al., 1986. Briefly, DOPE and diacylated RGD were dissolved in chloroform and organic solvent was evaporated using rotary vacuum evaporator (Strike 102, Italy). A thin lipid film was formed which was hydrated using PBS (pH 7.4). Dispersion was vortexed strongly for some times. Dispersed liposomes were sonicated for 3 min to transform them in to small unilammelar liposomes. For the formation of SK encapsulated liposomes, streptokinase solution (1, 35,000 U/ml) was used as a dispersion media. The unentrapped drug from the prepared vesicular system was separated by gel filtration using Sephadex G-100.

Characterization of developed liposomes

The liposomal systems were characterized for size, size distribution, shape, entrapment efficiency, in vitro release study, platelet targeting potential and *in vitro* thrombolytic activity. To assess targeting potential of liposomes, platelets binding studies were performed using human platelets in a suspension and evaluated by fluorescence microscopy and FACS analysis. *In vitro* clot dissolving study was performed in sterile microcentrifuge tubes using platelet rich human blood clot.

Table 1: Characteristics of developed liposomes

DOPE: acylated peptide (mol/mol)	% EE	Vesicles size (nm)		PI
		Initial	After 24 hrs	
10:0.1	7±0.53	128±12	145±11	0.25±0.08
10:0.2	8±0.72	121±14	142±13	0.24±0.07
10:0.5	12±0.41	112±11	125±08	0.21±0.07
10:1.0	18±0.35	108±09	115±08	0.18±0.05
10:1.2	17±0.28	107±07	116±10	0.17±0.06
10:1.5	9±1.15	120±14	143±13	0.25±0.09

RESULTS AND DISCUSSION

Liposomes were of 100-120 nm size and spherical in shape with unilammellarity (Table 1). *In vitro* drug release study showed that nearly 40% drug of the entrapped drug was released in 12 hr in the PBS (pH 7.4), however on incubation with activated platelet about 90% of drug was released within 45 min (Fig. 1).

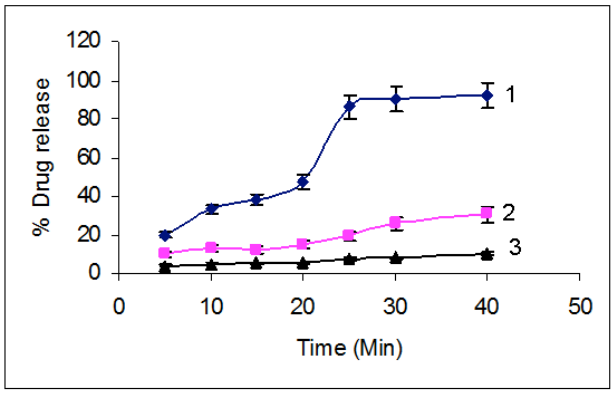

Fig. 1: *In vitro* release profile after incubation of RGD TS liposomes with platelets adhered and activated in the wells of 96-well microplate. RGD-liposomes (1) release drug in higher amount as compared to RAD liposomes (3) after interaction with activated platelets. RGD-liposomes incubated with resting platelets (2) release low amount of SK. Data are presented as mean±SD (n=5).

The results suggested target sensitivity of the liposomes. Further, targeting potential was confirmed by using microscopic study and flow cytometry. Thrombolysis study revealed that target sensitive liposomes could not only reduce the clot lysis time but also increased the extent of clot lysis as compared to plain streptokinase solution (Fig. 2 & 3).

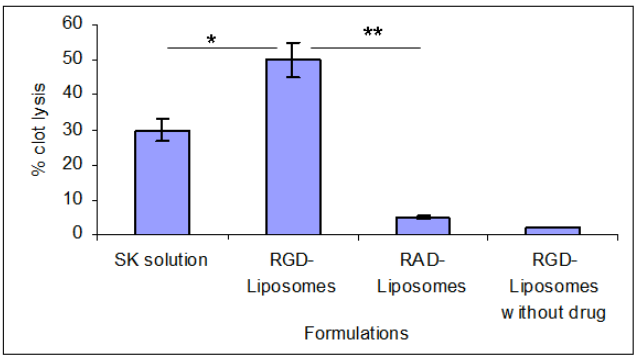

Fig. 2: Graphs showing % clot lysis with various SK formulations. % clot lysis for RGD TS liposomes are significantly higher (* p<0.01, n=3) than that of SK solution. % clot lysis for RAD liposomes are significantly lower than that of SK solution (p<0.001, n=3) and RGD liposomes (p<0.001, n=3).**

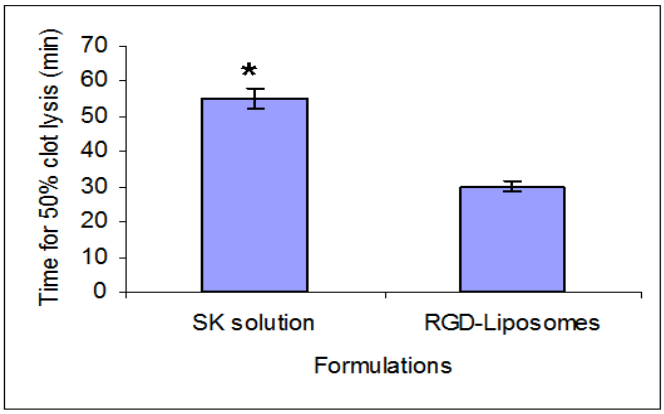

Fig. 3: Graphs showing clot lysis time (t50), time to dissolve 50% of clot wt, with various SK formulations. Clot lysis time for RGD TS liposomes are significantly shorter (* p<0.01, n=3) than that of SK solution.

CONCLUSIONS

Encapsulation in the liposomes increases the stability of SK in the plasma during *enroute* circulation by preventing their exposure to degrading substances. Developed RGD modified target sensitive liposomes was observed to release the drug at the target site (thrombus) after binding with the platelets embedded in the thrombus matrix while drug release in the circulation was retarded. The present liposomal formulation will targeting thrombolytic agent to the site of thrombus and hence will improve the therapeutic indices by simultaneous minimization of the side effect.

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