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Review article

Liposomal nanocarriers for plasminogen activators

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ABSTRACT

Several plasminogen activators (PAs) have been found effective in treating different thromboembolic diseases. However, administration of conventional thrombolytic therapy is limited by a low efficacy of present formulations of PAs. Conventional treatments using these therapeutic proteins are associated with several limitations including rapid inactivation and clearance, short half-life, bleeding complications or non-specific tissue targeting. Liposome-based formulations of PAs such as streptokinase, tissue-plasminogen activator and urokinase have been developed to improve the therapeutic efficacy of these proteins. Resulting liposomal formulations were found to preserve the original activity of PAs, promote their selective delivery and improve thrombus targeting. Therapeutic potential of these liposome-based PAs has been demonstrated successfully in various pre-clinical models in vivo. Reductions in unwanted side effects (e.g., hemorrhage or immunogenicity) as well as enhancements of efficacy and safety were achieved in comparison to currently existing treatment options based on conventional formulations of PAs. This review summarizes present achievements in: (i) preparation of liposome-based formulations of various PAs, (ii) development of PEGylated and targeted liposomal PAs, (iii) physicochemical characterization of these developed systems, and (iv) testing of their thrombolytic efficacy. We also look to the future and the imminent arrival of theranostic liposomal formulations to move this field forward.

Contents

Abbreviations: CDTs, clot dissolving times; Chol, cholesterol; CholS, cholesterol sulfate; DCP, dicetyl phosphate; DMPC, dimyristoyl phosphatidylcholine; DOPC, dioleoyl phosphatidylcholine; DOPE, dioleoyl phosphatidylethanolamine; DPPC, dipalmitoyl phosphatidylcholine; DPPG, dipalmitoyl phosphatidylglycerol; DSPE-PEG, distearoyl phosphatidylethanolamine — polyethylene glycol; DSPE-PEG-COOH, distearoyl phosphatidylethanolamine — carboxyl-polyethylene glycol; DSPE-PEG-MAL, distearoyl phosphatidylethanolamine — maleimide-polyethylene glycol; DSPC, distearoyl phosphatidylcholine; DRVs, dried-reconstituted vesicles; EM, electron microscopy; EMA, European Medicine Agency; FCL, fractional clot loss; FDA, Food and Drug Administration; GPIIb/IIIa, glycoprotein IIb/IIIa; IFVs, interdigitation-fusion of vesicles; i.v., intravenous; MGSVs, mean gray scale values; MPS, mononuclear phagocyte system; MSPC, myristoyl-stearoyl phosphatidylcholine; MI, mechanical index; MRI, magnetic resonance imaging; PAs, plasminogen activators; PAI-I/II, inhibitor of plasminogen activator I/II; PC, phosphatidylcholine; PE-MPB, phosphatidylethanolamine — maleimide-phenyl-butyramide; PEG, polyethylene glycol; PG, phosphatidylglycerol; POPC, palmitoyl-oleoyl phosphatidylcholine; PPP, platelet poor plasma; RAD, arginine-alanine-aspartate; REV, reverse-phase evaporation; RGD, arginine–glycine–aspartate; r-SAK, recombinant staphylokinase; rt-PA, recombinant tissue plasminogen activator; SAK, staphylokinase; SATA, succinimidyl-S-acetylthioacetate; SH, subconjunctivally induced hemorrhage; SK, streptokinase; t-PA, tissue plasminogen activator; u-PA, urokinase-type plasminogen activator; UK, urokinase; US, ultrasound.

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1. Introduction

Hemostasis represents a complex of normal physiological conditions preventing massive loss of blood after vascular injury and enabling for example demarcation of some infectious agent preventing their hematogenic spreading. Blood vessel injury triggers physiological coagulation processes that maintain the integrity of the circulatory system. Inappropriate activation of hemostatic mechanisms leads to pathological formation of a thrombus/clot (thrombosis). The pathogenesis of thrombosis is related to several abnormalities such as injury to vessel walls or endothelium damage caused through trauma or surgery, alterations in the rheology of normal blood flow induced by turbulent flow at bifurcations or by flow stagnation and changes in blood composition leading to hypercoagulability and hyperviscosity. There are two major constituents that form the thrombus network, namely activated platelets that produce a platelet plug and fibrin that forms a cross-linked matrix (Fig. 1) [1].

Inside blood vessels, a clot obstructs blood flow through the circulation and causes oxygen and nutrients deprivation to tissue leading to infarction. The clot can be formed in a place of occlusion or in some organs (e.g., heart or veins) and it can be also dislodged to become free-floating. Thereafter this traveling clot (embolus) is carried from such organs by blood flow through the circulatory system to various tissues (e.g., brain or lungs) eventually causing vessel obstruction or occlusion leading for example to stroke or pulmonary thromboembolism) [1].

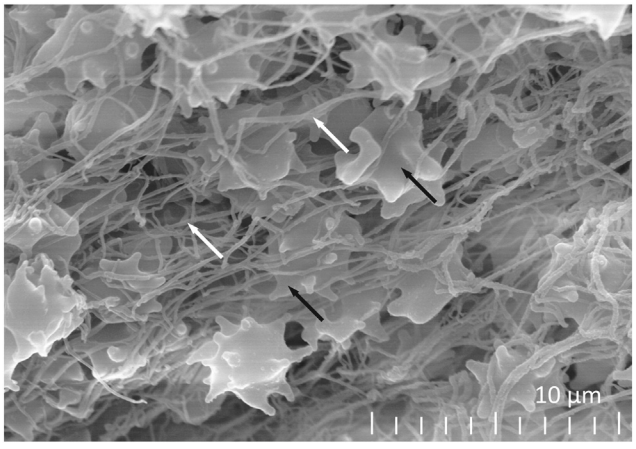

Fig. 1. The clot network formed by activated platelets (black arrows) and fibrin fibers (white arrows) observed by scanning electron microscopy.

2. Plasminogen activators

Various plasminogen activators (PAs) exist for clinical treatment of thromboembolic diseases. These include bacterial proteins such as streptokinase (SK), and serine proteases including urokinase-type plasminogen activator (u-PA), or tissue plasminogen activator (t-PA). PAs catalyze proteolysis of plasminogen at the arginine 561–valine 562 peptide link and so act to convert inactive plasminogen into active plasmin. The plasmin so formed triggers a fibrinolytic cascade that induces thrombolysis by degradation of fibrin present in the clot. The fibrin matrix can serve as both a plasmin substrate and as a surface for the specific adsorption of plasminogen or t-PA as well. Fibrinolytic activity in circulation is modulated by inhibitors of plasminogen activators (PAI-I, PAI-II) and inhibitors of plasmin (α1-antiplasmin, α2-macroglobulin) [2].

One of the first PAs used for thrombolytic therapy was SK. SK does not exhibit intrinsic enzymatic activity but instead activates plasminogen indirectly. SK acquires plasminogen activation properties by complexation with plasminogen. Upon interaction, the SK-plasminogen complex so generated activates plasminogen conversion into plasmin. However, SK activates not only fibrin-bound plasminogen but also circulating plasminogen. This effect causes a systemic generation of plasmin and can result in unwanted bleeding complications. In addition, SK is a non-human protein with an immunogenic effect that is linked to SK-specific antibodies. Therefore, multiple thrombolyses using SK are restricted by protein immunogenicity [3]. An alternative PA based on microbial proteins is staphylokinase (SAK) [4]. For SAK, high fibrin affinity has been observed in contrast to SK. There is also urokinase (UK), which is a direct PA. The human origin of UK removes the problem of antigenic and pyrogenic properties. However, UK does not exhibit the same affinity for fibrin as SK. UK also activates both fibrin-bound and circulating plasminogen creating a serious risk of hemorrhage as well. Otherwise, one of the most popular therapeutic PA is t-PA in various forms. This t-PA is a direct PA with selectivity for fibrin and also exhibits minimal immunogenic effects. Importantly t-PA has a 100-fold higher affinity for fibrin-bound plasminogen in the presence of circulating plasminogen. In terms of manufacture, t-PA is produced in a recombinant form (rt-PA). For instance, alteplase, reteplase or tenecteplase all represent recently developed rt-PAs approved by the FDA for thrombolytic therapy. Desmoteplase represent a novel potent PA under clinical trials. Currently, there are only PA-based forms which reached the stage of clinical testing or FDA approval for thrombolytic therapy. Generally, the developed PAs demonstrate short blood half-lives, e.g. 5, 10 and 30 min for t-PA, UK and SK, respectively [5]. Rapid renal clearance of the free proteins is standard due to the hydrophilic character and low molecular weight of the proteins, so too is the threat of enzymatic degradation in blood during passage through liver, spleen and kidneys. These factors significantly reduce the circulatory half-lives of PAs. Dosage application for a particular PA was summarized by Baruah et al. [5,6].