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ECHOGENIC LIPSOMES FOR TARGETED DRUG DELIVERY

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Abstract

Echogenic immunoliposomes (ELIP) are under development to enable ultrasound-controlled drug delivery. Mechanistic studies *in vitro* have revealed that stable cavitation is correlated with enhanced recombinant tissue Plasminogen Activator (rt-PA) thrombolysis, yet strategies to optimize the occurrence of such bubble activity and avoid potential harmful bioeffects have yet to be identified. Stable cavitation is characterized by bubbles pulsating gently in response to the time-varying acoustic pressure in an ultrasound field. A review of *in vitro* sonothrombolysis studies utilizing commercial US contrast agent or echogenic liposomes loaded with rt-PA to nucleate stable cavitation will be presented. Strategies for the development of ultrasound-enhanced thrombolysis and drug delivery will be discussed.

Targeted echogenic immunoliposomes (ELIP) are under development to highlight thrombus or atheroma components. Liposomes, or phospholipid bilayer vesicles enclosing gas and fluid, are novel agents that may permit evaluation of vasoactive and pathologic endothelium. Since first examined by Bangham *et al.* [1], liposomes have been developed for a wide range of technical and medical applications. Echogenic liposomal dispersions can be prepared by dispersing a lipid in water, adding mannitol, and lyophilizing [2-4]. The echogenicity of these preparations is due to the presence of gas, which is entrapped and stabilized by the lipid during the rehydration process following lyophilization [5].

ELIP can be targeted to certain tissues by attaching specific ligands and antibodies to the surface of liposomes [6,7]. For example, ELIP coupled to anti-VCAM-1 antibodies could be used to identify pathologic endothelium at early stages of atherosclerosis development. Similarly, the linkage of a liposome with antifibrin or D-Dimer antibody may identify and highlight thrombus or plaque rupture [8]. Lanza *et al.* [9] have also demonstrated that a multi-step acoustic biotinylated, lipid-coated, perfluorocarbon nanoemulsion could be successfully targeted to thrombi *in vitro* while maintaining ultrasound contrast. They have additionally demonstrated that this ultrasound agent can infiltrate arterial walls and localize tissue factor expression [10]. Unger *et al.* [11,12] created targeted microbubbles containing perfluorobutane (aerosomes) that were approximately 2 microns in diameter and were able to bind to thrombus *in vitro*. Echogenic liposomes, additionally, have the potential to encapsulate a drug for targeted therapeutic delivery.

Providing efficient and safe methods for the delivery of drugs to target cells is the principal goal of a clinically useful pharmacotherapeutic strategy. When a pharmaceutic is administered systemically, only a small fraction of the drug may reach the target tissue, necessitating large systemic doses to achieve effective local concentrations. Hence, systemic toxicity is usually the dose-limiting factor [13]. Localized pharmacotherapeutic delivery has distinct advantages, which include increased concentrations at the specific tissue site and decreased toxicities [14]. Direct delivery of a drug to the target tissue results in a high ratio

of local to systemic bioavailability, with the result of a decreased required total dose. As such, specific delivery may result in increased tolerable concentrations of the drug. In addition, the use of ultrasound to fragment all of the liposomes simultaneously near the target, rather than relying on a more gradual passive therapeutic delivery, has the potential to produce a large temporal peak in drug or therapeutic effect. This is particularly important at the endothelium where the constant flow of blood may carry away the released drug rapidly, making it unavailable for ultrasound-induced uptake across the endothelium.[15]

Additional benefits of liposomes are that lipids are small molecular structures and the lipid complexes can be made smaller by filtering or sonication techniques. Proteins and delivery agents made of proteins tend to break if they are manipulated to make them smaller. The difference lies in the rigidity of the protein, which has covalent bonds, *versus* the lipid, which is composed of small molecules held together by hydrophobic interactions. An example would be the rigidity of mayonnaise (a lipid formulation) *vs*. a hard-boiled egg (a protein formulation). Liposomes are ideal targeted delivery systems as they are nontoxic and can carry either hydrophilic or hydrophobic compounds, either in the aqueous compartment or within the phospholipid bilayers, respectively, and can be targeted to specific tissues. Hence, the development of a liposome-drug delivery mechanism that can be manipulated for site-specific tissue targeting may provide improved specificity of drug delivery and more efficient and enduring effects at the target site.

Several studies have demonstrated that low intensity (< 2 W/cm^2) ultrasound used as an adjuvant to recombinant tissue Plasminogen Activator (rt-PA), a thrombolytic, can increase thrombus dissolution in an *in vitro* model [16,17]. Significant enhancement of thrombolysis correlates with the presence of stable cavitation [18] and this type of gentle bubble activity, can be sustained using an intermittent infusion of a commercial contrast agent, Definity® [19]. In addition, inertial cavitation, which elicits broadband acoustic emissions, has been shown to be counter-productive for enhanced thrombolysis. Rather, the most effective form of bubble activity is stable cavitation, which elicits ultrasonic subharmonic generation. Increased penetration of rt-PA and plasminogen has been observed in clots exposed to stable cavitation nucleated by Definity®.

The thrombolytic, rt-PA, has been successfully loaded into ELIP and Smith *et al.* [20] have quantified ultrasound-triggered release of rt-PA in an *in vitro* flow model. These rt-PA-loaded ELIP are robust and echogenic during continuous fundamental 6.9-MHz B-mode imaging using a clinical diagnostic scanner at a low exposure output level (MI = 0.04). Furthermore, a therapeutic concentration of rt-PA can be released from the ELIP with pulsed 6.0-MHz color Doppler ultrasound at an MI above 0.43 [20]. Tiukinhoy *et al.* [21] and Klegerman *et al.* [22] have demonstrated specific fibrin binding of rt-PA-loaded ELIP using an *in vitro* porcine clot model. In addition, Shaw *et al.* have shown that the lytic efficacy of rt-PA-loaded echogenic liposomes is comparable to that of tPA alone [23]. Exposure of these ELIP to pulsed 120 kHz ultrasound (0.35 MPa peak to peak pressure amplitude, 50% duty cycle, 1667 Hz pulse repetition frequency) significantly enhanced lytic treatment efficacy for both rt-PA and rt-PA-loaded ELIP. These drug-loaded vesicles have the potential to be used for ultrasound-enhanced thrombolysis in the treatment of acute ischemic stroke, myocardial infarction, deep vein thrombosis, or pulmonary embolus.

Acknowledgments

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REFERENCES

1. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. Journal of Molecular Biology 1965;13:238–252. [PubMed: 5859039]
2. Alkan-Onyuksel H, Demos SM, Lanza GM, Vonesh MJ, Klegerman ME, Kane BJ, Kuszak J, McPherson DD. Development of inherently echogenic liposomes as an ultrasonic contrast agent. Journal of Pharmaceutical Sciences 1996;85:486–490. [PubMed: 8742939]
3. Huang SL, Hamilton AJ, Nagaraj A, Tiukinhoy SD, Klegerman ME, McPherson DD, Macdonald RC. Improving ultrasound reflectivity and stability of echogenic liposomal dispersions for use as targeted ultrasound contrast agents. Journal of Pharmaceutical Sciences 2001;90:1917–1926. [PubMed: 11745750]
4. Alkan-Onyuksel H, Demos SM, Kane B, McPherson DD, Klegerman ME. Echogenic immunoliposomes as an ultrasound imaging agent. ICAST Proceedings 1996:40–7.
5. Huang SL, Hamilton AJ, Pozharski E, Nagaraj A, Klegerman ME, McPherson DD, MacDonald RC. Physical correlates of the ultrasonic reflectivity of lipid dispersions suitable as diagnostic contrast agents. Ultrasound in Medicine and Biology 2002b;28:339–348. [PubMed: 11978414]
6. Martin, FJ.; Heath, TD. Covalent attachment of proteins to liposomes. In: RRC, N., editor. Liposomes: a practical approach. IRL Press; New York: 1990.
7. Leonetti J, Machy P, Degols G, Lebleu B, Leserman L. Antibody-Targeted Lipsomes Containing Oligodeoxyribonucleotides Complementary to Viral RNA Selectively Inhibit Viral Replication. Proceedings of the National Academy of Sciences April 1;1990 87:2448–2451. 1990.
8. Hamilton A, Rabbat M, Jain P, Belkind N, Huang SL, Nagaraj A, Klegerman M, Macdonald R, McPherson DD. A physiologic flow chamber model to define intravascular ultrasound enhancement of fibrin using echogenic liposomes. Investigative Radiology 2002;37:215–221. [PubMed: 11923644]
9. Lanza GM, Wallace KD, Scott MJ, Cacheris WP, Abendschein DR, Christy DH, Sharkey AM, Miller JG, Gaffney PJ, Wickline SA. A novel site-targeted ultrasonic contrast agent with broad biomedical application. Circulation 1996;94:3334–3340. [PubMed: 8989148]
10. Lanza GM, Abendschein DR, Hall CS, Scott MJ, Scherrer DE, Houseman A, Miller JG, Wickline SA. In vivo molecular imaging of stretch-induced tissue factor in carotid arteries with ligand-targeted nanoparticles. Journal of the American Society of Echocardiography 2000;13:608–614. [PubMed: 10849515]
11. Unger EC, McCreery TP, Sweitzer RH, Shen D, Wu G. In vitro studies of a new thrombus-specific ultrasound contrast agent. American Journal of Cardiology 1998;81:58G–61G.
12. Unger EC, Lund PJ, Shen DK, Fritz TA, Yellowhair D, New TE. Nitrogen-filled liposomes as a vascular US contrast agent: Preliminary evaluation. Radiology 1992;185:453–456. [PubMed: 1410353]
13. Groothuis DR. The blood-brain and blood-tumor barriers: A review of strategies for increasing drug delivery. Neuro-oncol January 1;2000 2:45–59. 2000. [PubMed: 11302254]
14. Eccleston DS, Horrigan MC, Ellis SG. Rationale for local drug delivery. Seminars in interventional cardiology : SIIC 1996;1:8–16. [PubMed: 9552480]
15. Hitchcock KE, Sutton JT, Caudell DN, Pyne-Geithman GJ, Klegerman ME, Huang S, Vela D, McPherson DD, Holland CK. Delivery of targeted echogenic liposomes in an ex vivo mouse aorta model. J Acoust Soc Am 2009;125:2713.
16. Holland CK, Vaidya SS, Datta S, Coussios CC, Shaw GJ. Ultrasound-enhanced tissue plasminogen activator thrombolysis in an in vitro porcine clot model. Thromb Res 2008;121:663–73. [PubMed: 17854867]
17. Meunier JM, Holland CK, Lindsell CJ, Shaw GJ. Duty Cycle Dependence of Ultrasound Enhanced Thrombolysis in a Human Clot Model. Ultrasound in Medicine and Biology 2007a;33:576–583. [PubMed: 17337113]
18. Datta S, Coussios CC, McAdory LE, Tan J, Porter T, De Courten-Myers G, Holland CK. Correlation of cavitation with ultrasound enhancement of thrombolysis. Ultrasound in Medicine and Biology 2006;32:1257–1267. [PubMed: 16875959]

19. Datta S, Coussios C-C, Ammi AY, Mast TD, de Courten-Myers GM, Holland CK. Ultrasound-enhanced thrombolysis using Definity® as a cavitation nucleation agent. Ultrasound in medicine & biology. 2008 In Press.

20. Smith DAB, Vaidya S, Kopechek JA, Hitchcock KE, Huang SL, McPherson DD, Holland CK. Echogenic liposomes loaded with recombinant tissue-type plasminogen activatory (rt-PA) for image-guided, ultrasound-triggered drug release. Journal of the Acoustical Society of America 2007b;122:3007.

21. Tiukinhoy-Laing SD, Buchanan K, Parikh D, Huang S, Macdonald RC, McPherson DD, Klegerman ME. Fibrin targeting of tissue plasminogen activator-loaded echogenic liposomes. Journal of Drug Targeting 2007a;15:109–114. [PubMed: 17365281]

22. Klegerman ME, Zou Y, McPherson DD. Fibrin-targeting of echogenic liposomes with inactivated tissue plasminogen activator. J Liposome Res. 2008a In press.

23. Shaw GJ, Meunier JM, Huang SL, Lindsell CJ, McPherson DD, Holland CK. Ultrasound-enhanced thrombolysis with tPA-loaded echogenic liposomes. Thromb Res. 2009 In Press.