Rationale for a Nanomedicine Approach to Thrombolytic Therapy

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Stroke is the third-leading cause of death and most common etiology for long-term adult disability. Of the approximate 700,000 individuals experiencing new or recurrent stroke annually, less than 5% of these individuals receive thrombolytic therapy. Intravenous recombinant tissue plasminogen activator (rt-PA) is an approved pharmacological treatment for acute ischemic stroke, but the window of opportunity is considered to be 3 hours from the onset of symptoms following a diagnostic imaging study to rule out hemorrhage. At the thrombolytic dosages approved, the incidence of intracranial hemorrhage in Europe and North America has been 6.4%. More recently, this time-window guideline has been liberalized to 3 to 6 hours, primarily because very few patients present for treatment within 3 hours. This change could bring a potential increase in the complication rate. The potential impact and value of a nanomedicine approach to this problem becomes apparent after examination of the alternatives that have been explored.

Neuroprotection
Neuroprotective approaches to stroke treatment have generated considerable interest because of the low effective use rate of thrombolytic therapies and, if successful, would extend the therapeutic window. However, hundreds of compounds and thousands of articles have been written on neuroprotecting agents to be administered in the advent of a cerebral vascular accident, but none has achieved regulatory-approved status. The evidence from PET studies has revealed that early reperfusion, either spontaneous or chemically initiated, primarily determines the final size of brain infarction; these hypoperfused regions account for 70% of the final infarct volume.

Small Molecule Thrombolytics
Reperfusion of the ischemic brain is the most effective therapy for acute stroke, restoring nutrition and oxygen-rich blood flow to threatened tissues. Endogenous enzymes such as rt-PA and urokinase control the conversion of plasminogen to plasmin and the ultimate digestion of fibrin. A number of circulating proteins neutralize plasmin; the best known is α-2-antiplasmin inhibitor, which forms a 1:1 stoichiometric complex with the bioactive β-chain of plasmin. This natural lytic cascade is tempered by a negative-feedback loop where plasmin reduces the expression of rt-PA and increases the production of plasminogen activator inhibitor (PAI)-1, which blocks the activity of rt-PA. A variety of novel fibrinolytic agents offer greater fibrin specificity, longer half-life, and increased resistance to PAI-1 inactivation than rt-PA. These include tenecteplase, desmoteplase, reteplase, plasmin, and microplasmin. Although tenecteplase, desmoteplase, reteplase all have improved pharmacokinetic properties or diminished side-effect profiles, none clearly provides a substantial improvement over tPA with regard to safety or efficacy. Plasmin and microplasmin, a truncated form of the bioactive β-chain of plasmin, act directly on fibrin but are rapidly inactivated by circulating antiplasmin. Plasmin and its derivatives have great potential for stroke revascularization, but only if delivered locally through an interventional radiology procedure in a hospital setting.

Sonothrombolysis
Sonothrombolysis is an experimental technique where ultrasound is applied to enhance enzymatic thrombolysis alone or in the presence of microbubbles. The combined treatment is associated with higher rates of recanalization. In the presence of microbubbles, higher rates of clot lysis have been associated with an increased incidence of cerebral hemorrhage, secondary to the intravascular cavitation of the bubbles and the mechanical rupture of contrast filled capillaries. In CLOTBUST, a phase II multicenter randomized trial, ultrasound in combination with rt-PA did induce recanalization or dramatic clinical improvement in 49% of treated versus 29% of control patients. Sonothrombolysis may offer medical advantage within the hospital setting, but the risks associated with rt-PA and/or equivalent lytic is not reduced and possibly increased by the potential for cerebral hemorrhage secondary to capillary rupture induced by microbubble cavitation.

Perfluorocarbon Nanoparticle Approach
Although incremental improvement will occur through improved responsiveness and education of the public and emergency management technicians, perhaps the best strat-
ergy is to ensure that eligible patients presenting to hospitals are treated with thrombolytic therapy. This goal will be greatly facilitated by reducing the complexity and risk of existing thrombolytic therapy approaches. We hypothesize that a nanomedicine approach may effectively address this critical issue.

Perfluorocarbon nanoparticles, previously considered as artificial blood substitutes, have been developed into a platform technology for molecular imaging and targeted drug delivery, ie, a so-called “theranostic” technology. These lipid-encapsulated particles, which are nominally 250 nm in diameter and administered intravenously, are constrained by size to the intact vasculature, as well as in the leaky neovascularizations associated with cancer, atherosclerotic disease, and inflammatory arthritis. In 1996, we were the first to demonstrate the in vivo use of these nanoparticles for fibrin-specific ultrasonic contrast and have subsequently further functionalized the surface with a variety of homing ligand and imaging complexes for MRI, nuclear, and optical applications. We proposed that fibrin-targeted perfluorocarbon nanoparticles, each presenting hundreds of fibrinolytic enzymes on the surface, could be administered easily as an IV rider over ∼10 minutes. The particles would circulate to the intraarterial thrombus and begin to recanalize the obstruction with the generation of plasmin intimately approximated to the fibrin fibrils. The particle-bound fibrinolytic activity would be constrained to the circulation, minimizing hemorraghic complications from extravascular enzyme diffusion. Whereas the concentration of enzymatic activity on the clot surfaces will accelerate and facilitate thrombus dissolution, the generation of plasmin by the particles is expected to be less than that of the equivalent free drug, because of the altered kinetics of plasminogen association with the tethered kinase enzymes. Finally, and importantly, the dosage of fibrinolytic activity given would be at least 10 to 100 times less than that typically given for the free enzyme.

We have reported that streptokinase can be covalently bonded to the surface of a perfluorocarbon nanoparticle, which can be targeted to clots and affect their rapid dissolution in vitro. Despite the restrictive binding of streptokinase nanoparticles to the clot surface, plasmin generated by the conversion of the proenzyme plasminogen penetrated the interstitium and affected rapid fibrinolysis. Samples treated with fibrin-targeted streptokinase nanoparticles in the presence of plasminogen exhibited a near-total loss of volume over the course of 60 minutes of exposure, whereas control groups whose treatment lacked either streptokinase or plasminogen remained effectively unchanged. The fibrinolytic effectiveness of the targeted streptokinase agent was reliant on enzymatic degradation of the fibrin matrix without additional mechanical destruction. This implies that the utility of this nanoparticulate thrombolytic agent in vivo would not be constrained by the need for external acoustic insonification for activation.

Fibrinolytic Nanoparticles: The Next Steps

The use of streptokinase to demonstrate the effect in vitro is unsuitable in vivo for preclinical animal models because of species-specific activity and in humans from an immunogenicity perspective. Although one might consider the ideal lytic enzyme to be rt-PA or an analog thereof, this was excluded because these molecules require fibrin binding for activation, which would leave most of the surface coupled enzyme ineffective. Instead, urokinase has been chosen and ongoing studies have optimized the ratio of surface urokinase to homing ligand, a murine monoclonal antibody specific for fibrin. Initial studies in vitro and in vivo have confirmed the effectiveness of this agent for both thrombolysis and thrombus homing (J.N. Marsh, etc, unpublished data, 2010). The next phases of the program will be to develop a nonmonoclonal antibody approach to targeting, which is in progress using phage display techniques, and to develop an enzyme-stabilization approach to ensure the activity of the agent for a minimum of 1 year under refrigeration. Achievement of these 2 milestones will permit a formal 2-year preclinical development pathway to a phase I clinical study. Based on the work to date, we anticipate that the dose of urokinase to patients administered as a short IV infusion will be <10% of the current loading dose used for the lytic systemically without the additional need for a continuous maintenance dose over 12 hours, making the total drug exposure <1%.

Summary

The current resistance of physicians to treat eligible patients presenting with ischemic stroke must be ameliorated to reduce significantly the severe mortality and morbidity anticipated for the US over the coming years. The unfortunate failure of neuroprotective technologies and the present risks and complexity associated with fibrinolytic therapy compels invention of new approaches to reestablish blood flow in stroke patients that are safer and simpler to use. We have proposed and demonstrated in vitro and in vivo a nanomedicine concept that will be simple to administer and that lowers the total dose of thrombolytic exposure. The nanoparticles and the lytic payload are anticipated to be vascularity constrained based on existing data, which will abrogate hemorrhagic complications attributable to extravascular dissolution of fibrin deposits from recent bleeds. However, future studies will need to demonstrate how the nanoparticle biodistribution and vascular constraint determined in neovascular dependent lesions, such as cancer and atherosclerotic plaque, will translate for ischemic brain tissue. A further advantage of this approach is the relatively rapid clearance of unbound fibrinolytic nanoparticle complexes from the circulation by the reticuloendothelial cells of the liver and spleen, which serve to neutralize the excess enzymatic activity. Although the development of this nanotechnology is nascent, the fundamentals of the approach suggest a great opportunity to favorably impact the large segment of undertreated stroke victims.

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