The Enhancing Effect of Focused Ultrasound on TNK-Tissue Plasminogen Activator-Induced Thrombolysis Using an In Vitro Circulating Flow Model

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Background: The limited efficacy of thrombolytic therapy in patients with ischemic stroke has created the need to use focused ultrasound (FUS) energy as a way to enhance thrombolysis efficacy (sonothrombolysis). Using an in vitro circulating flow model, we evaluated the role of physical parameters on tenecteplase (TNK-tPA)-mediated thrombolysis. *Materials and Methods:* Fully retracted porcine blood clots were used for the proposed experimental study. To provide a more realistic clinical environment of stroke, the study was conducted under realistic flow conditions and TNK-tPA concentrations. Two spherically FUS transducers (4-cm diameter), focusing at 10 cm and operating at .6 and 1.05 MHz, respectively, were used. Pulsed ultrasound protocols that maintained a localized temperature elevation at the focus of 1°C were applied. Thrombolysis efficacy was measured in milligram of mass clot removed. *Results:* The effect of physical parameters such as temperature, FUS frequency, acoustic power (AP), FUS energy, and microbubble (MB) administration on thrombolysis efficacy was examined. *Conclusions:* Study findings established that higher FUS frequencies (1 MHz) are associated with enhanced thrombolysis compared to lower FUS frequencies (.6 MHz). Furthermore, an increase in the linear relationship between AP and thrombolysis efficacy was exhibited. Also, the outcome of the study showed that the combination of 1-MHz FUS pulses with MBs strongly enhanced the enzymatic thrombolytic efficacy of TNK-tPA, because with 30 minutes of treatment, 1050 mg of clot was removed through nonthermal mechanisms. Taking into consideration that stroke is time dependent, this thrombolytic rate should be sufficient for timely recanalization of the occluded cerebral artery. **Key Words:** TNK-tPA—stroke—thrombus—ultrasound.

Introduction

Stroke is a leading cause of morbidity and mortality worldwide.[1] According to the World Health Organization, each year 15 million people worldwide suffer a stroke and of these, nearly 6 million die and another 5 million are left permanently disabled.[2] Approximately 87% of the annual incidences of stroke are ischemic.[3] Acute ischemic stroke (AIS) is the sudden obstruction of the blood flow to the brain, caused by a blood clot, which rapidly leads to severe brain tissue damage. If the cerebral artery is not opened quickly, the ischemic process get worse, leading to tissue death and cerebral infarction Therefore, the key of ischemic stroke treatment is the rapid restoration of blood flow in the occluded cerebral artery.[4]

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However, at present, intravenous (IV) thrombolysis with recombinant tissue plasminogen activator (rt-PA), which has been established as the only thrombolytic therapy approved in Europe and by the Food and Drug Administration in the United States, remains the most beneficial proven intervention for emergency treatment of stroke.[5] If rt-PA is given within 4.5 hours from symptom onset, then it can increase patient outcome.[6] However, less than 5% of patients with AIS receive IV rt-PA.[7] Of these, arterial recanalization is achieved in only 30%-40% and the recanalization is complete and sustained in only 18%.[8-10] As a result, many patients are left with a substantial brain damage, with high rates of disability and mortality.[11] Furthermore, rt-PA treatment involves a major risk of symptomatic brain hemorrhage.[12]

Because thrombolytic treatment is often not sufficient for timely vascular recanalization,[13] new therapeutic strategies that aim to improve recanalization rates and clinical outcomes after AIS are needed. A promising strategy, which represents an important breakthrough, is the application of ultrasound (US) energy to improve the action of thrombolytic drugs. US-enhanced thrombolysis, or sonothrombolysis, is a new and promising therapy for the treatment of AIS, in which the effectiveness of thrombolytic drugs can be increased when combined with transcranial US.[14-16] Many in vitro[17,18] and in vivo[19,20] studies have shown that US energy accelerates thrombolysis due to a possible increase in the enzymatic effect of thrombolytic drugs.

Although the mechanisms involved in sonothrombolysis are not fully understood, it is hypothesized that US energy

a) promotes motion of fluid around the clot surface (microstreaming), leading to increased delivery of the rt-PA near occlusion,[21,22] and
b) weakens fibrin cross-links, leading to increases in uptake, penetration, and concentration of rt-PA to the binding sites of the clot.[23,24]

In addition, the administration of gaseous microbubbles (MBs), that is, US contrast agents, may further increase US-enhanced rt-PA-induced thrombolysis, according to many in vitro[25,26] and in vivo[27,28] studies. MBs oscillate, expand, or collapse when exposed to a US field, producing either stable cavitation (microstreaming) or inertial cavitation (microjetting). Stable cavitation is associated by sustained bubble activity, which agitates the fluid where the spheres are dissolved, improving rt-PA delivery and penetration inside the clot.[29,30] Inertial cavitation is associated by a rapid increase in the size of bubbles, followed by a violent collapse, which can cause mechanical fragmentation of the thrombus.[31,32]

During the last decade, focused ultrasound (FUS) has emerged as a relatively new approach to sonothrombolysis. Focused transducers are used to transmit high pressures noninvasively into millimeter-sized focal volumes with little power deposition occurring outside the focal volume. Therefore, this novel therapeutic method enables noninvasive treatment of clots that causes no permanent damage to the surrounding tissue. FUS has been used in combination with rt-PA in vitro[33,34] and in vivo[35,36] to improve clot lysis efficiency over both rt-PA alone and FUS alone.

Despite the encouraging results of the preclinical studies, the results from clinical trials are not very optimistic. In the past decade, there have been some significant clinical trials concerning the application of therapeutic US in the treatment of stroke.

One of the first clinical trials on sonothrombolysis was the Transcranial Low-Frequency US-Mediated Thrombolysis in Brain Ischemia (TRUMBI), which was designed to treat stroke patients with either rt-PA alone or with 300-kHz US plus rt-PA.[37] The study was stopped prematurely due to the very high rate of symptomatic intracranial hemorrhage in patients treated with US and rt-PA. Since then, low-frequency US has not been available for therapeutic purposes in clinical trials.

The safety of higher frequencies (2 MHz) was studied using low-intensity US (similar to that used for diagnostic purposes), in the Combined Lysis of Thrombus in Brain Ischemia Using Transcranial Ultrasound and Systemic rt-PA (CLOTBUST) trial.[38] Study results have shown that the combination of rt-PA with 2 hours of continuous transcranial Doppler increased recanalization rates compared with rt-PA alone.

In another clinical trial, patients with proximal middle cerebral artery (MCA) occlusion were treated with 1.8-MHz transcranial color-coded duplex technology in association with rt-PA.[39] Study results also showed improved recanalization rates when transcranial color-coded duplex was combined with rt-PA in patients with AIS.

More recently, clinical research has been focused on the boosting effect of MBs to enhance sonothrombolysis. In 2009, Molina et al performed the Transcranial Ultrasound in Clinical Sonothrombolysis (TUCSON) clinical trial.[40] In Molina et al's study, stroke patients were treated with 2-MHz transcranial Doppler in combination with rt-PA and continuous infusion of MBs (MRX-801) (ImaRx Therapeutics, Inc., Tucson, Arizona). Although 50% reperfusion enhancement was detected at a low dose of MBs, when the dose was doubled, reperfusion enhancement ceased and hemorrhaging occurred. As a result, the clinical trial terminated, showing that further research in the field of MB-enhanced sonothrombolysis has to be done.

The objective of the present study was to evaluate the use of pulsed FUS exposures to improve tenecteplase (TNK-tPA)-mediated thrombolysis using a circulating pulsatile flow model. The outcome of the study could potentially be developed for clinical application such as the treatment of stroke patients. Because the most common cause of stroke is the occlusion of MCA or one of its branches, the physiological situation of MCA branch occlusion was reproduced. For this purpose, porcine blood clots were exposed to a circulating pulsatile flow and were treated with pulsed FUS in combination with TNK-tPA. The study evaluated the