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● *Original Contribution*

ULTRASOUND-INDUCED THERMAL ELEVATION IN CLOTTED BLOOD AND CRANIAL BONE

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Abstract—Ultrasound thermal effects have been hypothesized to contribute to ultrasound-assisted thrombolysis. To explore the thermal mechanism of ultrasound-enhanced thrombolysis with recombinant tissue plasminogen activator (rt-PA) for the treatment of ischemic stroke, a detailed investigation is needed of the heating produced in skull, brain and blood clots. A theoretical model is developed to provide an estimate for the worst-case scenario of the temperature increase in blood clots and on the surface of cranial bone exposed to 0.12- to 3.5-MHz ultrasound. Thermal elevation was also assessed experimentally in human temporal bone, human clots and porcine clots exposed to 0.12 to 3.5-MHz pulsed ultrasound *in vitro* with a peak-to-peak pressure of 0.25 MPa and 80% duty cycle. Blood clots exposed to 0.12-MHz pulsed ultrasound exhibited a small temperature increase (0.25° C) and bone exposed to 1.0-MHz pulsed ultrasound exhibited the highest temperature increase (1.0° C). These experimental results were compared with the predicted temperature elevations. (E-mail: nahirnvm@email.uc.edu) **© 2007 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasound, Hyperthermia, Thrombolysis.**

INTRODUCTION

Recent studies of ultrasound-enhanced thrombolysis have indicated the possibility of using lower exposure levels (<2.5 W/cm^2) in combination with thrombolytic drugs for restoration of blood flow in the arteries in the heart, legs and brain (Rosenschein et al. 1997; Atar 2001; Atar et al. 2001; Atar and Rosenschein 2004; Pfaffenberger et al. 2005; Alexandrov et al. 2004). Also, lower-intensity therapeutic ultrasound applications are under investigation to promote gene transfection and enhanced drug delivery (Bekeredjian et al. 2003; Taniyama et al. 2002).

Recombinant tissue plasminogen activator (rt-PA) is moderately effective in lysing thrombi in ischemic stroke patients and improves neurologic deficits if given within three hours after the onset of stroke symptoms (Wolpert et al. 1993). Unfortunately, thrombolytics also can cause intracerebral hemorrhage. Thus an adjuvant therapy that lowers the systemic dose of rt-PA or increases its thrombolytic efficacy would represent a significant breakthrough.

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Effective methods of enhancing thrombolysis have been examined in an attempt to reduce the dosage of the thrombolytic agent and reduce the risk of hemorrhagic events. Kudo (1989) extended the use of therapeutic ultrasound to increase the efficacy of systemic rt-PA. They delivered transcutaneous 200-kHz continuous wave ultrasound to enhance rt-PA–induced fibrinolysis in a canine femoral arterial thrombus model. Lauer et al. (1992) demonstrated that 1-MHz intermittent ultrasound, with an exposure interval of 2 s followed by a quiescent interval of 2 s, increased the percent mass loss in a whole human blood clot model *in vitro*. They proposed that acoustic streaming alone, without cavitational effects, was responsible for the increased thrombolysis. Careful investigations by Francis and his coworkers suggest that ultrasound accelerates enzymatic fibrinolysis by increasing transport of reactants through a cavitation-related mechanism (Francis et al. 1992; Blinc et al. 1993; Francis et al. 1995). However, experiments using ultrasound exposure of clots in a hyperbaric chamber revealed that other mechanisms in addition to inertial cavitation were present (Everbach and Francis 2000).

Several investigators have utilized transcranial, low-frequency, low-intensity ultrasound to accelerate thrombolysis (Akiyama et al. 1998; Behrens et al. 1999; Shaw et al. 2001a, 2001b) and Holland et al. (2002)

provided experimental evidence *in vitro* that ultrasound can be used in combination with rt-PA to increase thrombus dissolution in porcine and human clot models. Suchkova et al. (2002) explored ultrasound-enhanced fibrinolysis at low kilohertz frequencies (27–100 kHz) in an attempt to minimize ultrasonic heating and concomitant adverse bioeffects (McDannold et al. 2004; Duckett et al. 2004; Vella et al. 2003). In addition, clinical trials conducted by Alexandrov et al. (2004) have shown efficacy of ultrasound-enhanced thrombolysis in the treatment of ischemic stroke in patients.

Mild heating of only a few degrees can increase the enzymatic activity of rt-PA and can contribute to the enhanced thrombolysis (Shaw et al. 2006). The rates of particle diffusion and biochemical reactions are both temperature dependent (Cheng 1981). Also, an increase in temperature can affect membrane permeability, active transport processes and metabolic rates (Nyborg and Ziskin 1985). Enzymatic biochemical reaction rates increase with rising temperature to a specific temperature threshold, where the enzyme becomes denatured and the reaction rate subsequently declines (Cheng 1981). Thus one would expect that a thermal mechanism could contribute to enhanced thrombolysis in the presence of ultrasound hyperthermia.

Sakharov et al. (2000) and Shaw et al. (2003) have explored the contribution of enhanced thrombolysis as a result of mild hyperthermia in the absence of ultrasound exposure. The effects of a 6° C temperature rise above 37° C on the lytic rate in a human plasma clot model were measured *in vitro* by Sakharov et al. (2000). A 6° C temperature increase caused a doubling in the percent lysis after a 30-min exposure to this elevated temperature. Similarly, Sakharov et al. (2000) explored the contribution of stirring on the observed enhanced thrombolysis. Sakharov et al. (2000) hypothesized that the acceleration of enzymatic plasma clot lysis as a result of ultrasound exposure was caused by the effects of both heating and acoustic streaming. However, neither of these ultrasonic effects was observed or measured directly. Because the thermal effects do not account solely for the observed enhancement of thrombolysis, many authors have concluded that this enhancement is the result of a combination of mechanical and thermal mechanisms (Dick et al. 1998; Francis et al. 1992; Lauer et al. 1992; Blinc et al. 1993; Harpaz et al. 1993; Olsson et al. 1994; Sakharov et al. 2000).

In this study, we explore the extent of heating with ultrasound parameters in the range used for ultrasound-enhanced thrombolysis studies (Holland et al. 2002; Shaw et al. 2001a, 2001b; Alexandrov et al. 2004) by deriving an analytic model of hyperthermia in clotted blood exposed to 0.12-MHz, 1.0-MHz and 3.5-MHz pulsed ultrasound. For such a model to be developed, the acousto-mechanical and thermal properties of clotted blood must be well characterized. We rely on the previous study of the acousto-mechanical and thermal properties of clotted blood as inputs for this model (Nahirnyak et al. 2005, 2006).

The investigation of the transcranial transmission losses through a human skull demonstrated mild attenuation (22.5%) of the ultrasound beam at 120 kHz (Coussios et al. 2002). A larger amount of heating at the surface of the cranial bone would be expected at higher frequencies. To investigate this concern, we performed numerical estimates for the temperature rise on the front face of human skull using the results of the theory developed by Nyborg (1988).

The thermal elevation was also assessed experimentally in clotted blood and human temporal bone exposed to pulsed ultrasound *in vitro* with the same range of center frequencies (0.12 MHz, 1.0 MHz and 3.5 MHz). These experimental results were compared with the predicted temperature elevation in clotted blood and bone.

METHODS

Theoretical model

Absorption of ultrasound can cause a temperature increase in soft tissue and bone. By knowing the intensity of ultrasound in the tissue and the physical properties of the surrounding material, it is possible to calculate theoretically the expected thermal elevation in both soft tissue and cranial bone for the *in-vitro* case investigated in this work. For this purpose we need, namely, the coefficient of acoustic absorption, density, specific heat and thermal conductivity of clotted blood and cranial bone.

To determine the spatial and temporal dependence of temperature elevation $T(\vec{R}, t)$ in our experimental blood clots exposed to pulsed ultrasound, we chose to solve the bio-heat transfer equation for an idealized spherical tissue insonified by plane waves (see Figs. 1 and 2). The bio-heat transfer equation in the tissue characterized by thermal conductivity K, density ρ and specific heat, C_m, ignoring perfusion, is given by the following equation (Nyborg 1988):

$$\nabla_r^2 T - \frac{1}{\kappa}\frac{\partial T}{\partial t} = -\frac{2\alpha I}{K} = -4\pi\,\Psi(\vec{r}, t), \qquad (1)$$

where $\kappa = \dfrac{K}{\rho C_m}$ is the thermal diffusivity of tissue, α is the amplitude absorption coefficient in the blood clot, I is the spatial average, temporal average intensity in the incident ultrasonic wave, $\Psi(\vec{r}, t)$ is the heat source function, $\vec{r}$ is the radius vector from the center of the clot and t is time. Only a radial term appears in the expression for the Laplacian operator, because in our model we consider the clot as an absorbing sphere of radius a, where