Simulation of Intracranial Acoustic Fields in Clinical Trials of Sonothrombolysis

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Abstract - Two clinical trials using sonothrombolysis to improve rt-PA thrombolysis in patients with acute ischemic stroke have been carried out. .The CLOTBUST trial reported accelerated recanalisation by monitoring the middle cerebral artery (MCA) in patients with symptoms compatible with MCA infarction using 2-MHz transcranial Doppler ultrasonography. In CLOTBUST there was no increased bleeding as evidenced by cranial computer-tomography. The TRUMBI trial, which employed meticulous MRI imaging before and after rt-PA thrombolysis, was discontinued prematurely because of an increased number of secondary hemorrhages, possibly related to the use of low frequency 300-kHz ultrasound. The purpose of our work is to help identify possible mechanisms of intracerebral hemorrhage resulting from sonothrombolysis by applying a simulation tool that estimates the pressure levels in the human brain that are produced with different sonothrombolysis devices. A finite difference time domain (FDTD) scheme was developed to predict acoustic pressures in the brain. This tool numerically models the wave propagation through the skull and reproduces the two ultrasound protocols of CLOTBUST and TRUMBI for analysis of the distribution of acoustic pressure in the brain during treatment. For the simulated CLOTBUST trial, the peak negative pressure is about 1 bar corresponding to an MI in the brain of less than 0.07. This simulated pressure is below the cavitation threshold, but likewise far lower than any acoustic pressure that has been reported as sufficient for effective sonothrombolysis. For the simulated TRUMBI trial, the peak negative pressure in the brain is higher than the cavitation threshold in large areas of the brain, even outside the targeted clot. This is mainly due to the presence of standing waves and of constructive interferences. Simulating the pressure field of ultrasound protocols for clinical trials of sonothrombolysis may help explain mechanisms of adverse effects. Such simulations could prove useful in the initial design and optimization of future protocols for this promising therapy of ischemic stroke.

Key Words: stroke, ultrasound, sonothrombolysis, numerical simulation, safety

INTRODUCTION AND LITERATURE

Stroke is now the second leading cause of death in industrialized countries. The great majority of strokes are ischemic (85%). This is generally due to a blood clot, which occludes a cerebral artery and causes brain necrosis in the territory of the supplying vessel. The key point in the treatment of ischemic stroke is the rapidity of the reperfusion to avoid irreversible damage of brain tissue and sequelae for the patient (Caplan 1999). So far, intravenous recombinant tissue plasminogen activator (tPA) is the only effective and approved treatment available for acute ischemic stroke, despite controversial results concerning the balance between its established beneficial thrombolytic effect and its potential neurotoxicity (Benchenane et al. 2004). Several *in vitro* and *in vivo* (animal models) studies revealed that exposure of clots to low-frequency (from 20 kHz to 2 MHz), low-intensity (from 700 mW/cm² to 1.75 W/cm²) ultrasound accelerates the effect of fibrinolytic agents (Kudo 1989; Lauer et al. 1992; Kornowski et al. 1994; Francis et al. 1995; Behrens et al. 1999; Daffertshoffer et al. 2004). The mechanisms implied are non-thermal effects (Blinc et al. 1993). It is thought that ultrasound-induced perturbation of the clot (acoustic streaming, stable cavitation) creates exposure of additional binding sites for t-PA. All experimental studies performed using animal models (Daffertshoffer et al. 2004) found no harmful secondary effects due to ultrasound insonification. However, the therapy of ischemic stroke is highly complex because the disease damages the blood vessels walls, disturbing the fragile equilibrium of the blood brain barrier. In addition, the injection of plasminogen activators may increase the rate of hemorrhagic transformation through proteolytic and anticoagulation pathways. Thus, a promising approach to treatment of acute stroke would be to reduce the concentration of t-PA for clot lysis through enhancement of the thrombolytic effect with ultrasound. However, the exact mechanisms involved in the dissolution of the thrombus under ultrasound exposure remains unclear (Francis and Suchkova 2001). Two clinical trials of

sonothrombolysis have been reported recently with varying results: the CLOTBUST and the TRUMBI trials. The purpose of our work is to better understand the pressure levels involved in those studies through numerical simulations.

In 2004, Alexandrov's team led a multi-center randomized clinical trial called CLOTBUST (Combined Lysis of Thrombus in Brain Ischemia Using Transcranial Ultrasound and Systemic t-PA) (Alexandrov et al. 2004). This study was performed on 126 patients who had acute ischemic stroke due to occlusion of the middle cerebral artery (MCA). All patients were treated with intravenous t-PA within three hours after the onset of symptoms. 63 of them received pulsed 2-MHz-ultrasound through transcranial Doppler ultrasonography, and 63 constituted the control group and received placebo. The emitter was a 10mm-diameter single element delivering a 2-MHz pulsed wave with duty cycle of 15% and short emission duration of 15 μs. The focusing was set in the ipsilateral hemisphere. The focal length depends on the targeted region: proximal (between 30mm and 45mm) or distal (more than 45mm). The derated Spatial Peak Time Averaged Intensity I_{SPTA} was 739 mW/cm². In the target group, ultrasonographic monitoring began before t-PA injection and was continued for two hours. After two hours, a complete reperfusion or dramatic clinical recovery was observed for 49% of the patients in the target group (t-PA+US) and for only 30% of the control group. The study was not powered to evaluate patient outcome after three months, which was not statistically different for the two groups. No secondary effects linked with ultrasound exposure were identified.

On the other hand, another clinical study on sonothrombolysis for ischemic stroke in humans was stopped prematurely because of the occurrence of a higher number of intracerebral hemorrhages after combined t-PA treatment with transcranial sonication at 300 kHz (Daffertshofer et al. 2005). The TRUMBI (Transcranial Low-Frequency Ultrasound-Mediated Thrombolysis in Brain Ischemia) trial was carried out on 26 patients. 12 patients

received standard t-PA treatment (0.9 mg/kg) and 14 were treated with t-PA and transcranial insonation of low-frequency pulse-wave mode ultrasound. The diamond pattern transducer (50-mm diameter) emitted an unfocussed pulsed ultrasound wave at 300-kHz modulated in frequency (± 1.5 kHz) to limit standing waves. The targeted zone was the contralateral brain hemisphere corresponding to a distance around 100-mm.

The emission parameters reported by Daffertshofer (2005) include a 5% duty cycle (corresponding to an emission duration of 500 μs), and a spatial-peak time average intensity (I_{SPTA}) of 700 mW/cm² in water with a pulse repetition frequency of 100 Hz. Taking into account the duty cycle, the spatial-peak pulse-average intensity (I_{SPPA}) was 14 W/cm^2, corresponding to a mechanical index (MI) in water of 1.18. However, the authors claim that the mechanical index was less than 0.2. Wang (2007) recently estimated the MI used in the TRUMBI study and concluded that the value given by Daffertshofer (2005) was wrong: the MI was 1.18 and the pressure was higher than the one expected by the authors of the TRUMBI study. However, a closer look at the description of the emission parameters given in Daffertshofer et al. (2005) could be interpreted in another way: the 5% duty cycle has been added in order to 'further reduce thermal effects' and was thus not taken into account in the evaluation of the 700 mW/cm² I_{SPTA} in water, so that technically speaking it corresponds to an I_{SPPA} of 700 mW/cm². This corresponds to the back-calculation of a mechanical index of 0.25. Since it is unclear which interpretation of the published data is correct, both parameters will be evaluated in this study:

- a high hypothesis that an I_{SPPA} of 14W/cm² was used in the TRUMBI study (as supposed by Wang et al (2007))
- a low hypothesis that an I_{SPPA} of 700 mW/cm² was used in the TRUMBI study as supported by the designers of the TRUMBI device.

Regardless of which explanation is correct, it will be shown that both hypotheses lead to the same conclusion: the acoustic pressure in the brain outside the clot was high enough to induce stable cavitation and BBB opening at undesired locations.

In the TRUMBI study, ultrasound exposure was applied simultaneously with a t-PA injection for 90 minutes. Unlike the CLOTBUST study, however, rigorous MRI monitoring was performed in all patients and not just in those where control imaging after thrombolysis was warranted clinically. The TRUMBI study was stopped because MRI evidence of haemorrhage was observed for 93% of the target patients and 42% of the control patients. Among these hemorrhages, all were classified as hemorrhagic transformation in the t-PA only group versus 61% in the t-PA plus ultrasound group. Five haemorrhages in the target group were symptomatic hemorrhages, possibly linked to ultrasound exposure.

MATERIALS AND METHODS

Several in vitro studies have suggested that stable cavitation – bubble formation in the rarefactional pressure zones – is an important mechanism of sonothrombolysis (Blinc et al. 1993; Everbach and Francis 2000; Behrens et al. 2001; Meunier et al. 2007). We studied the pressure amplitude distribution in the brain to localize potential areas of acoustic cavitation. The simulations were performed with a software based on a finite differences scheme developed in the Laboratoire Ondes et Acoustique (UMR CNRS 7587, Université Paris 7) (Aubry et al. 2003).

The skull acoustical model

From high resolution computed tomography (CT) images, a 3D portion of the skull is reconstructed. As previously described in (Aubry et al. 2003), the acoustical properties of the skull can be deduced from the raw CT data.

The brain acoustical model

The brain is a soft tissue, with an acoustical behaviour close to that of water (Wells 1977):

$$\rho_{\text{brain}} = 1000 \text{ kg/m}^3;\ c_{\text{brain}} = 1500 \text{ m/s}. \quad (1)$$

The brain is an absorbing medium, the absorption coefficient depends on the ultrasound frequency and was set to (Goss et al. 1978) .

$$\text{abs}_{\text{brain}} = 0.05 \text{ dB/mm/MHz}. \quad (2)$$

Simulation Code

3D finite simulations have been performed in order to evaluate pressure field distributions and pressure levels in the brain. Simulations were performed with a finite differences program called Acel. Acel is a C++ numerical code developed at the Laboratoire Ondes et Acoustique. It solves the 3D linear wave equation in heterogeneous and absorbing media:

$$\left(1 + \tau_0(\vec{r})\frac{\partial}{\partial t}\cdot\right)\left[\rho_0(\vec{r})\nabla\cdot\left(\frac{1}{\rho_0(\vec{r})}\nabla p(\vec{r},t)\right)\right] - \frac{1}{c_0(\vec{r})^2}\frac{\partial^2 p(\vec{r},t)}{\partial t^2} = S_0(\vec{r},t) \quad (3)$$

where $c_0(\vec{r})$ is the speed of sound, $\rho_0(\vec{r})$ the density and $\tau_0(\vec{r})$ the absorption coefficient in the medium.

Simulations were conducted at a 2MHz and 300kHz central frequency. The simulation grid was set to one tenth of a wavelength. In order to meet the stability criteria, the temporal step is given by $\Delta t < \frac{\Delta x}{\max_{\vec{r}}(c_0(\vec{r}))\ \sqrt{3}}$, where Δx is the spatial step of the grid. An example of the 3D pressure field in the region of interest located in front of the transducer is given on Figure 1. For sake of clarity, only 2D views will be displayed in the results.

Figure 1

The Mechanical Index (MI)

In order to evaluate the likelihood of cavitation-related biological effects, the most relevant indicator was established to be the Mechanical Index (MI) (Apfel and Holland 1991). Indeed acoustic cavitation activity occurs in areas of rarefactional pressure and depends on the ultrasound frequency. The MI is defined as follows:

$$\mathrm{MI} = \frac{P_-}{\sqrt{f}}. \tag{4}$$

with P_- the peak rarefactional pressure in megapascal and *f* the ultrasound center frequency in megahertz.

In this study, the MI related to each clinical trial is deduced from the I_{SPTA}.

$$\mathrm{I_{SPTA}} = P_-^2 / 2\rho c, \tag{5}$$

ρ is the mass density and c is the ultrasound waves velocity. Assuming that the non-linear effects are negligible in this configuration, we considered

$$P_{p2p} = 2P_-, \tag{6}$$

P_{p2p} is the peak-to-peak acoustic pressure.

To avoid adverse biological effects related to acoustic cavitation, the FDA (Food & Drug Administration - 510K Norm 1992) imposes the diagnostic devices to ensure a MI less than 1.9 (Dalecki 2004).

No adverse biologic effect induced by ultrasound alone on mammalians has been recorded for MI<0.3 (AIUM/NEMA 1992). Nevertheless this threshold may be lowered in the presence of tPA and clot and in standing wave field.

The MI takes into account only the frequency dependency and not the impact of the duty-cycle and other emission parameters, which can lead to the occurrence of biological effects.

RESULTS

The CLOTBUST study

According to Eq. 4 and Eq. 3, the derated $I_{SPTA.3}$ in Alexandrov's study (Moehring et al. 2000) corresponds to a peak negative pressure of $3.85.10^5$ Pa and a MI of 0.27 in water without crossing the skull.

The CLOTBUST configuration described in (Moehring et al. 2000; Alexandrov et al. 2004) has been simulated. A 10mm-diameter emitter sent a 2-MHz pulsed wave during 15 μs with a pulse repetition frequency of 10.2 kHz (15% duty cycle). The focal length was set to 45 mm.

The negative peak pressure recorded in the simulation was less than 1.10^5 Pa in the whole brain and was about $0.5.10^5$ Pa close to the clot. The MI in the brain was thus less than 0.07.

The TRUMBI study

A 2D-configuration of the TRUMBI study with a 50mm-diameter emitter has been simulated. The targeted zone was set to 100 mm from the probe in the contralateral hemisphere. The emitter was positioned in front of the temporal bone, either on the left or the right window. Table one summarizes the simulated pressure levels near the clot and the maximum pressure levels achieved in the brain (on the so-called 'hot spot') with the corresponding MI.

Table 1.

Several points are crucial to analyze the interaction between the biological medium and the ultrasound field: the location and the amplitude of the peak rarefactional pressure and the possibility of standing waves formation.

The emission duration in the TRUMBI protocol (500µs) corresponds to several crossings of the brain. The ultrasound wave is reflected more than four times which creates constructive and destructive interferences between the emitted wave and the reflected ones. The acoustic pressure level recorded at the “hot spot” and close to the clot (right temporal window) are shown in Figure 2 in the case of the high hypothesis. On the case of the low hypothesis, the patterns are identical and only the pressure amplitudes differ.

Figure 2

These interferences may produce “hot spot” far from the clot. The location of these “hot spots” is highly dependent on the skull shape and on the position of the probe.

Figure 3

Comparing the pressure amplitude crossing the brain through the right temporal window (Fig. 3.a)to the pressure amplitude through the left temporal window (Fig. 3.b), several differences may be noted. The peak rarefactional pressure recorded through the emission time is not located in the same area: between the emitter and the clot through the right side and near the bone at the opposite side from the emitter through the left side. In Figure 3.a, the acoustic beam is reflected with an angle whereas in Figure 3.b the reflected wave travels parallel to the incident wave. Hence, in the latter case, a standing wave is likely to appear. Figure 2.a illustrates an interesting phenomenon: the plane wave emitted by the transducer has been focussed towards the hot spot because of a lens effect induced by the shape of the skull.

This kind of effect must be carefully taken into account when designing a non focussed device for brain therapy.

DISCUSSION

Kudo (Kudo 1989) was one of the first to report the use of transcranial ultrasound supply to enhance the effect of thrombolytic agent (t-PA). Several studies have underlined the high sensitivity of this technique to ultrasound emission parameters: frequency, intensity, duty-cycle (continuous or pulsed waves), pulse duration. Some trends have been established (Atar et al. 1999) :

- the higher the frequency is, the higher the absorption of the skull (Fry and Barger 1978; Suchkova et al. 1998). As a consequence, the energy delivered by the transducers has to be higher and may induce heating of the temporal skin (Daffertshofer and Fatar 2002);
- the higher the intensity applied to the clot is, the faster is its dissolution (Blinc et al. 1993; Suchkova et al. 1998);
- the higher the duty cycle or the pulse duration is, the better is the lysis of the clot but it induces thermal effects and damaging temperature rises may be harmful (Rosenschein et al. 2000).

One of the main differences between the trials studied in this article is the ultrasound frequency. In the CLOTBUST trial, the frequency is seven times higher than in the TRUMBI trial, thus the attenuation through the skull is much higher and explains mostly the difference in the pressure level. Sonothrombolysis devices are often characterized by the value of their I_{SPTA} in water. Such systems should also provide the mean I_{SPTA} expected at the focus in the brain, as this data, combined with the duty cycle and pulse duration, represents more accurately the possible effects of ultrasound in the brain.

The critical issue as underlined by Francis and Suchkova (2001) is to identify the optimum combination between frequency, intensity and waveform to combine treatment enhancement and safety. To reach this goal a better understanding of the mechanisms induced by ultrasound exposure is needed. According to several in vitro studies (Blinc et al. 1993; Everbach and Francis 2000; Behrens et al. 2001; Meunier et al. 2007), stable acoustic cavitation seems to be the key process of low-frequency sonothombolysis. Nevertheless there is little evidence that this phenomenon occurs *in vivo* and one need to be cautious in extrapolating the *in vitro* results to the clinical situation. Using low energy, the purpose of this therapy is to avoid inertial cavitation and its thermal effects. The mechanisms by which ultrasound enhances enzymatic thrombolysis appear primarily related to enzyme transport into the clot. Stable acoustic cavitation is liable to create microstreaming which optimizes the contact between the thrombolytic drug and the clot, moreover the oscillations of the bubbles in contact with the clot may induce the disruption of fibrins of the clot (Francis and Suchkova 2001).

The conditions of bubble formation, the location where they are formed in the blood and how they interact with the blood brain barrier are not clear (Hajri et al. 2005). Nevertheless, even though the role of cavitation is not fully understood, the main ultrasound-induced mechanism seems to be stable acoustic cavitation (Blinc et al. 1993; Everbach and Francis 2000; Behrens et al. 2001; Meunier et al. 2007). The phenomenon occurs when the negative acoustic pressure in the medium goes beyond a pressure threshold.

The stable acoustic cavitation threshold applied to our analysis was derived from (Azuma et al. 2005). In Azuma's study the acoustic cavitation threshold was measured in degassed water, in a standing wave field established inside a human skull, at 617-kHz frequency. The peak rarefactional pressure was about 2.75 10^5 Pa corresponding to a MI of approximately 0.35 (Eq. 4). It is important to note here that the cavitation threshold in a standing wave field is much smaller than the threshold in a progressive wave field. This is

mainly due to the fact that nano-bubbles can be trapped in antinodes and create bigger bubbles by coalescing.

Figure 4 represents in grey the areas where the pressure went beyond the threshold calculated for the TRUMBI frequency ($P_{-} = 0.35\sqrt{0.3} = 0.19$ MPa - Eq. 4) during one emission cycle with the high hypothesis.

Figure 4

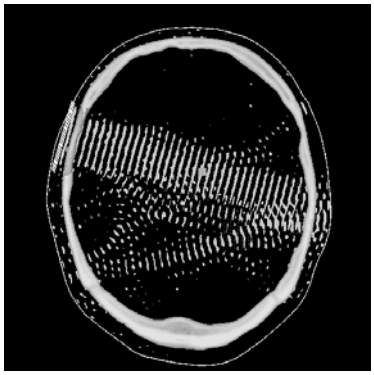
The zone where the acoustic cavitation is liable to take place is not confined to the clot. This observation may be related to the occurrence of atypical haemorrhages outside the targeted region. In the case of the low hypothesis, the maximum pressure amplitude is 0.27 MPa, also higher than the cavitation threshold (Fig. 4.a).

The analysis of simulated ultrasound propagation revealed that the reflected wave interferes constructively with the emitted one, increasing the pressure level. Three alternatives may be proposed:

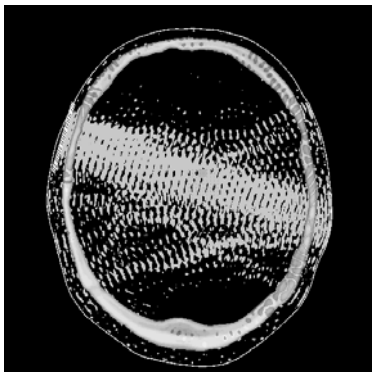
- to use a higher frequency to decrease the interferences as absorption in the brain will be higher
- to shorten the emission duration in order to avoid the constructive interferences between a reflected wave and the emitted one
- to apply a frequency modulation

Different frequency modulation options have been tested (Fig. 5): ramp or sinusoidal modulation with various frequency ranges. Areas where the acoustic pressure is higher than 0.19MPa (Azuma et al. 2005) are displayed in grey color. One can see that as frequency is shifted, the locations of the maximum pressure amplitude are shifted: nano bubbles are not trapped at the same location for a long time any more, but the pressure

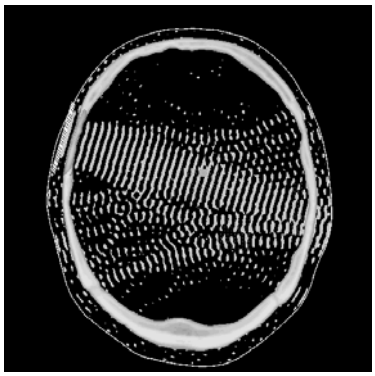
levels remains higher than 0.19MPa. Simulations help to appreciate the effect of such modulations.



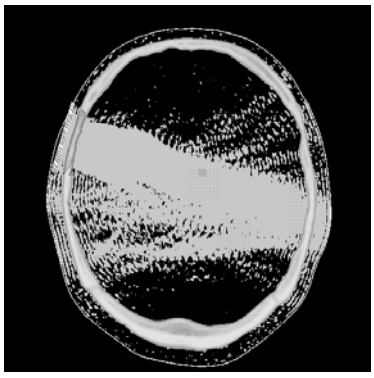
a) No modulation



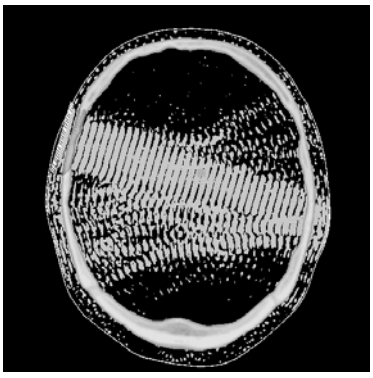
b) ± 1.5 kHz (ramp)



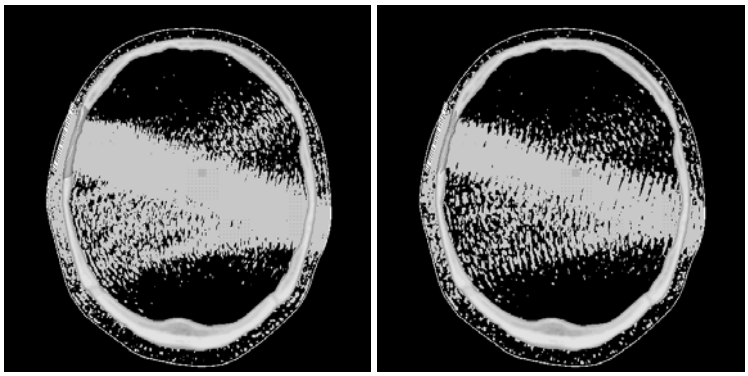
c) ± 1.5 kHz (sinus)



d) ± 15 kHz (ramp)



e) ± 15 kHz (sinus)



f) ± 150 kHz (ramp) g) ± 150 kHz (sinus)

Figure 1. Comparison of pressure repartition in different cases of frequency modulation

The acoustic stable cavitation areas presented in Figure 5 depend on the acoustic stable cavitation threshold that is chosen. As underlined by Vykhodtseva and colleagues (Vykhodtseva, Hynynen et al. 1995), it is very difficult to measure the stable and inertial cavitation thresholds in living tissues because they depend on numerous parameters: propagation medium, ultrasound frequency, duty cycle, pulse duration, standing wave….

In a recent paper (Datta, Coussios et al. 2006), Datta and colleagues demonstrated the sensitivity of cavitation thresholds to the medium. The likelihood of cavitational effects is higher when the ultrasound field is applied to plasma containing tPA and a clot, than when it is to plasma alone (cf Table 2). As a consequence it would be possible to apply an acoustic field inducing cavitation only at the clot location characterized by a pressure higher than the stable acoustic threshold in presence of a clot but lower than the stable cavitation threshold relative to the brain without a clot. The critical point is to define both thresholds: one for brain containing tPA and a clot P_{clot} and one for brain and tPA P_{noclot}, in order to know how to accelerate the clot dissolution without risking to damage the rest of the brain.

On another hand, Azuma et al have described and experimented the bubble-formation mechanism in a standing wave field (Azuma, Kawabata et al. 2005). This phenomenon is particularly tough near the bone at the border zone and could explain that secondary

haemorrhages occurred distant to the target point. This study confirms the influence of standing waves on cavitation phenomenon: in degassed water inside a skull the stable cavitation threshold was lower than in Datta's study where the medium was unconfined (Table 1).

	Frequency (MHz)	Peak rarefactional pressure for stable cavitation (MPa)	**MI**
(Azuma, Kawabata et al. 2005)			
Degassed water and skull	0.617	0.275	**0.35**
(Datta, Coussios et al. 2006)			
Degassed water	0.12	>0.65	**>1.88**
Plasma alone	0.12	0.39	**1.13**
Plasma + tPA	0.12	0.34	**0.98**
Plasma+tPA+clot	0.12	0.2	**0.58**

Table 1. Stable acoustic cavitation thresholds from (Azuma, Kawabata et al. 2005) and (Datta, Coussios et al. 2006)

Five haemorrhages of the t-PA plus ultrasound group in the TRUMBI trial were not classified as hemorrhagic transformation. Several hypotheses are formulated in Daffertshoffer's paper (Daffertshofer, Gass et al. 2005) and corroborate the observations and comments resulting from our simulation study. It seems that the shape of the human skull is liable to act as a lens and create some "hot spot" where cavitation takes place as previously shown in Figure 3.a. More precisely, one can notice in Figure 6 that the peak rarefactional pressure recorded is in the area where the atypical secondary haemorrhage occurred for the pathological case reported in (Daffertshofer, Gass et al. 2005), whatever the hypothesis. With

the high hypothesis the pressure is well above cavitation threshold, and with the low hypothesis, the pressure is slightly above cavitation threshold.

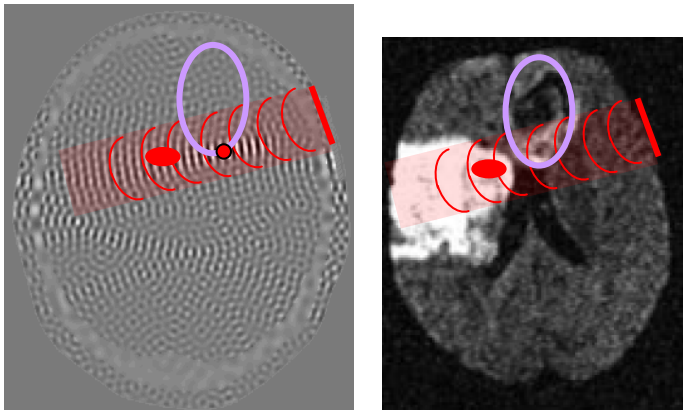


Figure 2. From Daffertshoffer et al.(Stroke 2005) – Figure 3. Localization of the clot and of the secondary hemorrhage compared to ultrasound field.

Thus, the curvature of the skull plays a role and may explain the discrepancy between the results of this study and the previous ones carried out *in vitro* or on animal models. In order to precisely analyze the reasons why some patients of the TRUMBI trial developed atypical haemorrhages, CT scans of their skull would be needed to carry out the simulations in the most realistic conditions.

A recent paper (Reinhard, Hetzel et al.) points out the abnormal permeability of the human blood-brain barrier which can be induced by wide-field low-frequency insonation. The observed excessive bleeding rate with low-frequency sonothrombolysis might thus be attributable to primary blood-brain barrier disruption by ultrasound.

Some studies have demonstrated that ultrasound exposure could open the blood-brain barrier (BBB). In (Hynynen, McDannold et al. 2006), the threshold for BBB disruption was approximately evaluated to 0.4 MPa, using 260 kHz focussed ultrasound bursts and ultrasound contrast agent. This threshold is lower than the stable acoustic cavitation threshold and lower than the maximum peak pressure amplitude obtained in the brain in our simulation of the TRUMBI setup, with both hypotheses (high and low).

Assuming that ultrasound induced BBB opening in areas remote from the brain infarction, tPA could diffuse in the brain parenchyma. As tPA is a known neurotoxic agent which can induce haemorrhages (Benchenane, Lopez-Atalaya et al. 2004; Kaur, Zhao et al. 2004), this might also explain the occurrence of secondary haemorrhages in TRUMBI. Nevertheless, the BBB disruption mechanism remains unclear.

The results of our simulations are likewise informative for the CLOTBUST study. The simulated maximum negative peak pressure is about 1 bar, far below the cavitation threshold at 2MHz: the simulated MI in the brain is less than 0.07. In fact, the pressure is so low after attenuation of the skull bone that it has been questioned whether any thrombolytic effect at all can be expected (Pfaffenberger et al., Stroke 2005 36(1) pp. 124-128. To our knowledge, all studies reported in the literature observed an accelerated clot lysis for acoustic pressure ranging from 1 to 4 bars for a frequency domain from 40 kHz to 2 MHz. The corresponding MIs ranged from 0.3 to 1.2, much higher than achieved in the CLOTBUST study (Lauer, Burge et al. 1992; Blinc, Francis et al. 1993; Harpaz, Chen et al. 1994; Kornowski, Meltzer et al. 1994; Francis, Blinc et al. 1995; Tachibana and Tachibana 1995; Akiyama, Ishibashi et al. 1998; Suchkova, Siddiqi et al. 1998; Behrens, Daffertshofer et al. 1999; Everbach and Francis 2000; Behrens, Spengos et al. 2001; Pieters, Hekkenberg et al. 2004; Datta, Coussios et al. 2006; Meunier, Holland et al. 2007). It would thus be interesting to perform *in vitro* tests with a MI close to the one used in the CLOTBUST study (0.07) to evaluate *in vitro* the degree of the acceleration of the clot lysis.

The main results are summarized in Table 3 with the high hypothesis for TRUMBI. It highlights the fact that I_{SPTA} alone cannot give an idea of the exact amount of pressure that will be delivered to the brain: the duty cycle is also important (doubles the expected pressure in water in this case). At higher frequencies, ultrasonic waves do not penetrate the skull easily, so that the simulated pressure is higher in the case of TRUMBI. Such an effect is

enhanced by the fact that longer emission signals were emitted in the case of TRUMBI, potentially increasing the maximum pressure level on hot spots due to constructive interferences.

	CLOTBUST	TRUMBI
Frequency	2MHz	300kHz
I_{SPTA}	739 mW/cm^2	700 mW/cm^2
Expected maximum pressure in water	3,85. 10^5 Pa	6,5. 10^5 Pa (×2)
Simulated maximum pressure in the brain	0,7.10^5 Pa	6.10^5 Pa (×10)

Table 2. **Simulation results for CLOTBUST and TRUMBI setups.**

CONCLUSION

The comparison of these two clinical trials shed a new light on the need for intensive investigations to better understand the mechanisms and bioeffects of sonothrombolysis. Our approach emphasizes that ultrasonic emission conditions (frequency, duty cycle and emission duration) are of fundamental concern to ensure the safety of the sonothrombolysis treatment. The application of ultrasound in the treatment of ischemic stroke seems to be a two-edged sword: very promising but very delicate. The critical issue is to identify the optimum combination between frequency, intensity and waveform to perform the safest and the most efficient treatment. Numerical tools provide us with a unique opportunity to explore different hypotheses, thus helping to find the optimal pressure level for safely applying the benefits of ultrasound exposure in stroke treatment. Namely, the use of focussed ultrasound should be preferred to unfocussed beams as hot spots outside the thrombus would be more easily avoided: one would prefer to suffer from a defocusing effect of the skull rather than a

focusing effect concentrating ultrasound at an unwanted location as shown in this study; and the expected pressure levels inside the brain should be evaluated numerically prior to human trails to better evaluate the potential risks. In the case of the TRUMBI study, two hypotheses have been investigated (high and low hypothesis): depending on the case, the pressure is well above or slightly above cavitation threshold outside the targeted region and pressure levels can induce BBB opening, putting the brain at risk with tPA extravasations.

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