Transcranial Low-Frequency Ultrasound-Mediated Thrombolysis in Brain Ischemia

Increased Risk of Hemorrhage With Combined Ultrasound and Tissue Plasminogen Activator

Results of a Phase II Clinical Trial

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Background—Clinical studies using ultrasound at diagnostic frequencies in transcranial Doppler devices provided encouraging results in enhancing thrombolysis with tissue plasminogen activator (tPA) in acute stroke. Low-frequency ultrasound does not require complex positioning procedures, penetrates through the skull better, and has been demonstrated to accelerate thrombolysis with tPA in animal experiments in wide cerebrovascular territories without hemorrhagic side effects. We therefore conducted the first multicenter clinical trial to investigate safety of tPA plus low-frequency ultrasound (300 kHz).

Methods—Acute stroke patients within a 6-hour time window were included (National Institutes of Health Stroke Scale scores >4). Magnetic resonance imaging (MRI) was used to document vascular occlusion and to rule out cerebral hemorrhage. Patients were allocated to combination therapy alternately; the first patient received tPA only, the second patient received tPA plus ultrasound, etc. Follow-up included serial MRI directly thereafter and 24 hours later to confirm recanalization and tissue imaging. Clinical recovery was measured after treatment and 3 months later.

Results—26 patients (70.4±9.7 years) entered the trial (12 tPA, 14 tPA plus ultrasound). The study was prematurely stopped because 5 of 12 patients from the tPA only group but 13 of 14 patients treated with the tPA plus ultrasound showed signs of bleeding in MRI ($P<0.01$). Within 3 days of treatment, 5 symptomatic hemorrhages occurred within the tPA plus ultrasound group. At 3 months, neither morbidity nor treatment-related mortality or recanalization rates differed between both groups.

Conclusions—This study demonstrated bioeffects from low-frequency ultrasound that caused an increased rate of cerebral hemorrhages in patients concomitantly treated with intravenous tPA. (***Stroke*. 2005;36:1441-1446.)**

Key Words: stroke, acute ■ thrombolysis ■ ultrasonography

Intravenous recombinant tissue plasminogen activator (tPA) is the only effective and approved treatment available to date for acute ischemic stroke.[1] However, only 11% more patients achieved a favorable outcome at 3 months compared with placebo while incurring an increased risk of symptomatic and asymptomatic intracerebral hemorrhage of 6.4%.

Experimental data suggest that exposure of an acute intracranial clot with ultrasound potentiates tPA–mediated thrombolysis in vitro and in vivo.[2,3] Ultrasound can induce various changes such as reversible disaggregation of cross-linked fibrin fibers,[4] microcavity formation in the shallow layer of thrombus,[5] and increasing uptake and penetration of tPA into clots.[6] Insonation through the skull is associated with a remarkable loss of energy at frequencies used for diagnostic purposes (2 to 4 MHz). This results in failure of clot degradation in experimental settings that involve temporal bone obstacles.[7] Using lower frequencies (20 kHz to 1 MHz), tPA–mediated clot degradation was as much as 50% more efficient when ultrasound was added,[2,8,9] even when applied transcranially.[8,10,11] In the thromboembolic middle cerebral artery, rat mode low-frequency ultrasound (185 kHz and 33 kHz) is more efficacious than the higher frequencies used in commercially available diagnostic devices.[9–11] The bleeding rate was similar in both groups (tPA alone versus tPA plus ultrasound), and there were no side effects on the blood–brain barrier.[11] The first multicenter randomized clinical study[12] using 2-MHz transcranial ultrasound probes suggested enhancement of tPA activity with acceleration of arterial reperfusion but so far no clinical improvement. Although encouraging, these data lack confirmation of vascular and brain tissue effects through gold

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TABLE 1. Inclusion and Exclusion Criteria

Inclusion Criteria
Acute onset of a focal neurological deficit (NIHSS >4 points)
Diagnostic MRI before tPA bolus to demonstrate
Absence of hemorrhage (T2*)
Ischemic lesion (DWI)
Vascular obstruction (MRA)
Intravenous tPA (0.9 mg/kg, 10% bolus 90% infusion over 1 hour, maximum dose 90 mg) within 0 to 3 hours (group A) and 3 to 6 hours (group B).
Exclusion Criteria
Primary intra-arterial thrombolysis
Patient refusal or inability to give informed consent
Evidence of intracranial hemorrhage on pretreatment evaluation, including SAH
Active systemic, internal bleeding
Known bleeding diathesis
Abnormal INR or PTT values.
Platelet count <100 000/mm3
Significant subcortical vascular encephalopathy
Complete MCA or hemispheric infarction
Known intracranial neoplasm, arteriovenous malformation, or aneurysm
Any neurological disease with neurological impairment interfering with the new acute symptoms
Blood pressure >220 mm Hg systolic or >120 mm Hg diastolic
Seizure at onset of stroke
Rapidly improving symptoms before treatment (at least 30 minutes persistence of symptoms)
Recent (within 3 months) intracranial surgery, trauma, or disabling stroke
Blood glucose of <50 mg/dL or >400 mg/dL
Any contraindications to perform MRI investigations
Age older than 85 years or younger than 18 years
Pregnancy

INR indicates International Normalized Ratio; PTT, prothrombin time.

standard imaging procedures and are in contrast to experimental studies using diagnostic ultrasound plus tPA.[7]

The major objective of this study was to test safety and practicability of thrombolytic therapy in acute stroke patients with combined application of tPA plus low-frequency ultrasound. A secondary objective was to compare clinical recovery and rates of recanalization, reperfusion, and infarct size as evidenced by serial MRI.

Patients and Methods

Transcranial Low-Frequency Ultrasound-Mediated Thrombolysis in Brain Ischemia (TRUMBI) was a phase II, prospective, nonrandomized, multicenter trial. Patients were alternately allocated a standard 0.9-mg/kg tPA treatment and a combination of tPA treatment with transcranial insonation of low-frequency pulse-wave mode ultrasound for 60 to 90 minutes.

Patients were divided into target and control groups. Target groups consisted of patients treated within the 3-hour (group A) and the 6-hour (group B) time window after stroke onset with standard intravenous tPA plus ultrasound. Control patients were treated within 3 hours (group C) and 6 hours after stroke onset (group D) with standard tPA only. A total of 48 patients were planned to enter the study (32 patients with additional ultrasound exposure; 16 patients with tPA only). Inclusion and exclusion criteria are given in Table 1. Safety measures were set as primary end points: incidence of symptomatic (clinical worsening >4 National Institutes of Health Stroke scale points) and asymptomatic cerebral hemorrhage within 24 hours, and death at 3 months after treatment. Effectiveness measures were set as secondary end points: (1) rate of recanalization during, directly after, and 6 and 24 hours after the end of tPA as demonstrated by MRA; (2) level of tissue perfusion deficit within 6 hours after treatment demonstrated by perfusion-weighted imaging; (3) infarct volume within 6 hours demonstrated by MR–diffusion-weighted imaging (DWI) after treatment; (4) neurological outcome (NIHSS) within 24 hours; and (5) outcome at 3 months (NIHSS, modified Rankin score).

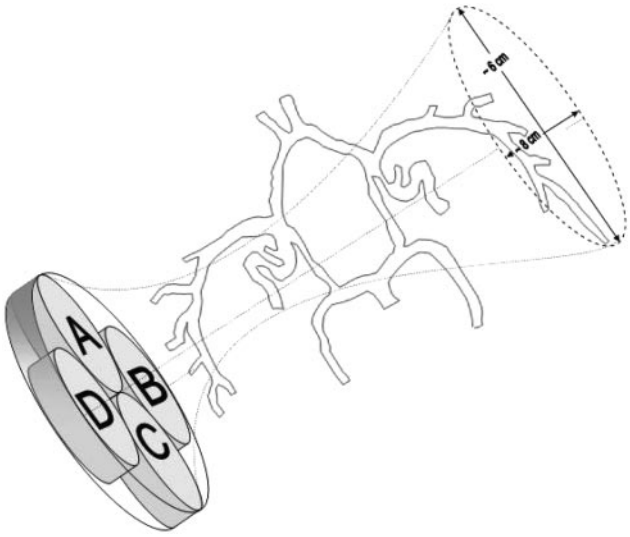

Figure 1. The transducer has 4 elements (A to D) arranged in a diamond pattern. These operated in pairs to insonify a particular region of the target anatomy. Thus, the CB pair or the CD pair (depending on the side of the head where the transducer is positioned) sonicate the circle of Willis area and the M1 segment. The DB pair covers the M2 and proximal M3 segment. The AD and AB pairs are targeted at the mid and distal M3 segments. The focus was set to the contralateral M1 segment (≈10 cm away from the probe surface). The effective ultrasound field around the beam axis at the sonicated target area covered ≈8 cm in frontal-occipital and ≈6 cm in cranio-caudal direction.

Neuroimaging

Pretreatment MRI protocol consisted of T1, T2, DWI, apparent diffusion coefficient (ADC), perfusion-weighted imaging, and T2* and time-of-flight MRA measures. Follow-up examinations included additional FLAIR measurements. MRI studies were reread by an independent imaging committee, and members were blinded to the assigned treatment. According to definitions published elsewhere,[13] hemorrhagic transformation (HT) was predefined as small petechiae along the margins of the infarct (HT1) or more confluent petechiae within the infarcted area but without space-occupying effect (HT2). Parenchymal hemorrhage (PH) was defined as blood clots with space-occupying effect. PHs were further classified into those that developed within the boundaries of cerebral infarction, which involved <30% of the infarcted area with mild mass effect (PH 1) and those that involved >30% of the infarcted area with significant mass effect (PH 2).[13] Vessel occlusion and recanalization were rated according to established criteria.[14] A CT scan was performed during days 3 to 7 after treatment to confirm MR classification of bleeding complications.

Ultrasound Treatment

Low-frequency ultrasound (300 kHz ±1.5 kHz to avoid standing waves) with an intensity of 700 mW/cm^2 (temporal average spatial peak intensity) was applied simultaneously with intravenous administration of tPA and for 30 minutes after tPA infusion (total insonation time=90 minutes). The transducer has 4 elements arranged in a diamond pattern

TABLE 2. Hemorrhages

	tPA Only MRI	Ultrasound Plus tPA MRI
None	7	1
HT1	3	6
HT2	2	2
PH2		2
HT1+SAH		1
PH2+SAH		1
PH1+HT1+VH		1
Total	12	14

VH indicates ventricular hemorrhage.

(Figure 1). The average temporal pressure was significantly <1 atmosphere, thereby avoiding cavitation. The mechanical index was <0.2. The low intensity levels, combined with the low frequency, resulted in a thermal index in soft tissue of <0.5 and a thermal index cranial of ≈4.0. The higher thermal index cranial was addressed through the use of a cooling pad and a thermal sensor to detect excessive heating. To further reduce thermal effects, ultrasound was emitted in a pulsed fashion with a 5% duty cycle and a pulse repetition frequency of 100 Hz (giving a cycle/pulse ratio of 225).

Statistical Analysis

The analysis was performed with the use of StatsDirect (version. 2.4.4) software (StatsDirect Ltd). Statistical significance for intergroup differences was assessed by the 2-tailed Fisher exact test and Pearson χ^2 test for categorical variables and Student *t* test and Mann–Whitney *U* test. A level of $P<0.05$ was accepted as statistically significant.

Results

Twenty-six patients were included in TRUMBI (12 control patients [group C n=8 and group D n=4]) with a mean onset to treatment time of 2.42±0.50 hours (range, 0.50 to 4.35 hours) and were treated with tPA only. Fourteen patients (group A n=10 and group B n=4) with a mean onset to treatment time of 2.45±1.03 hours (range, 0.30 to 4.25 hours) were treated with tPA plus ultrasound.

The study was stopped because of an increased number of hemorrhages on the follow-up MRI T2* imaging within 24 hours: 5 of 12 patients from the tPA only group and 13 of 14 patients treated with the combination of ultrasound plus tPA ($P<0.01$) showed some signs of intracranial hemorrhages. All hemorrhages in the tPA only group and 8 of 13 hemorrhages in the combined tPA plus ultrasound group were classified as hemorrhagic transformations (Table 2). Only in the combined ultrasound tPA group did we observe parenchymal hemorrhages (in the contralateral hemisphere in both cases) or hemorrhages in the subarachnoid (SAH) (2 cases) or ventricular spaces (1 case). Occurrence of hemorrhages was associated with the time to initiation of treatment in the treatment group as well as the control group (Table 3).

The number of hemorrhages identified on CT was considerably lower than from MRI T2* imaging. In the tPA only group, one HT was identified on CT among the 5 patients with MRI T2*-detected HT. In the target group treated with tPA plus ultrasound, only 1 HT was seen on CT among the 9 of 14 with MRI T2*-positive HT. Five of 14 patients with PH/SAH/ventricular hemorrhage (VH) on MRI T2* imaging had corresponding findings on CT.

Symptomatic bleeding associated with acute clinical worsening was observed in all of the 5 patients with PH/SAH/VH (all from the tPA plus ultrasound group). Significant adverse events during the 3-month follow-up occurred in 4 patients. One patient treated with tPA had a second stroke after only 6 hours. Three patients in the tPA plus ultrasound group died (one because of an atypical intracerebral hemorrhage 6 days after treatment in the

TABLE 3. Influence of Treatment Window on Bleedings and Recanalization

	No Bleeding or HT Only		PH or SAH or VH		*P*
All patients	21	159±58 min	5	186±49 min	<0.0001
tPA only	12	163±51 min	0	...	...
Ultrasound+tPA	9	154±70 min	5	186±49	<0.0001
	No Bleeding		Bleeding		
	No.	Time Window	No.	Time Window	
All patients	8	136±35 min	18	176±61 min	<0.0001
tPA only	7	139±37 min	5	195±53 min	<0.0001
Ultrasound+tPA	1	115	13	169±64	...
	Recanalization		No Recanalization		
	No.	Time Window	No.	Time Window	
All patients	16	155±54 min	10	170±59 min	<0.0001
tPA only	6	149±67 min	6	176±27 min	<0.0001
Ultrasound+tPA	4	163±35 min	10	167±73 min	<0.0001

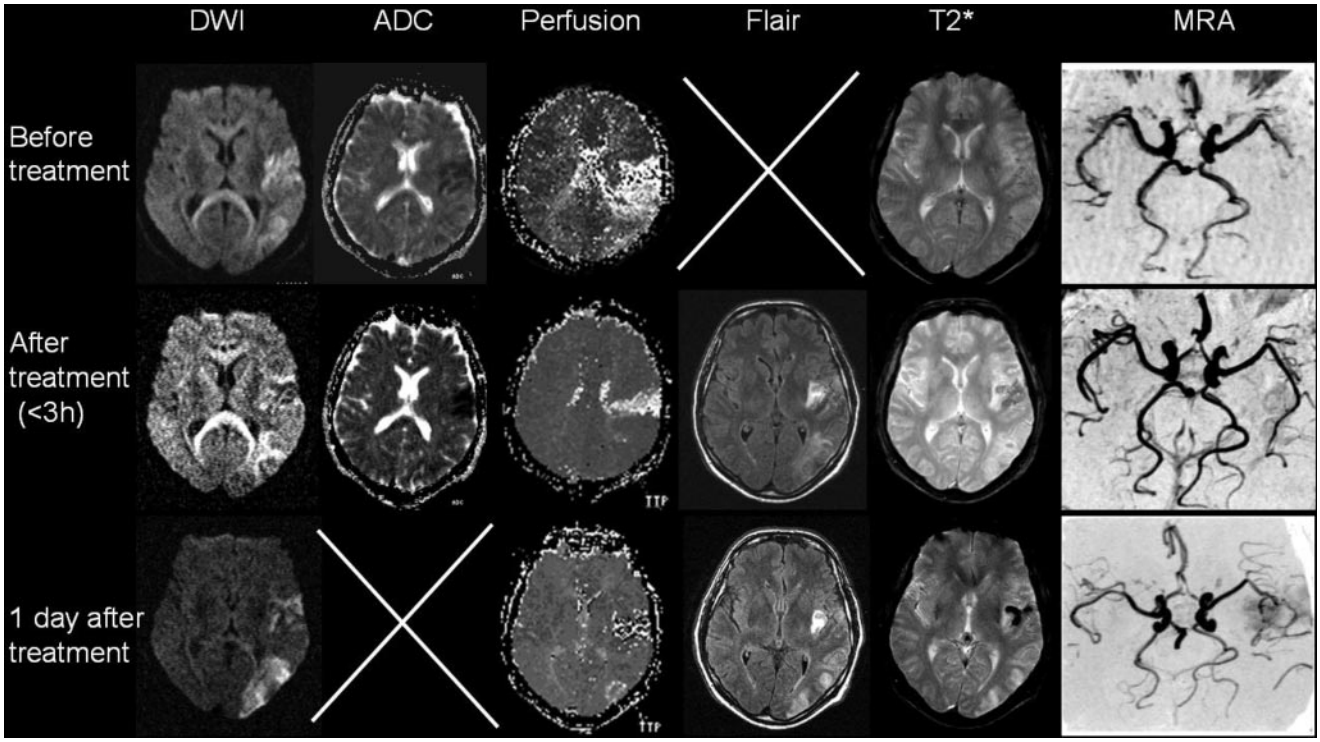

Figure 2. A 67-year-old patient with an acute right-side hemiparesis and aphasia (NIH 15). Treatment (tPA plus ultrasound) started 180 minutes after symptom onset. Pretreatment MRI (upper row) showed left hemispheric hypoperfusion extending the DWI/ADC deficit. MRA revealed occlusion within the M2 segment of the middle cerebral artery. Posttreatment MRI (middle row) displayed a decreasing territory of perfusion, but ADC abnormalities were almost unchanged. MRA showed reopening of the main M2 segment area, and some minor branches still seem to be occluded. Posttreatment NIH score was 8. Follow-up MRI 24 hours after symptom onset (lower row) showed residual infarction in DWI and FLAIR sequences. T2* imaging showed some subtle hemorrhagic transformation. At 3-month follow-up, NIH was 2, Barthel was 95, and Rankin was 1.

hemisphere contralateral to the ischemic infarction, one from myocardial infarction 3 days after treatment, and one from pulmonary embolism 34 days after treatment). Follow-up at 3 months revealed no significant differences in NIHSS (tPA only: 12±7; confidence interval [CI]: 4 to 12 versus tPA plus ultrasound: 14±9; CI, 6 to 18), Barthel Index (tPA only: 64±35; CI, 41 to 88 versus tPA plus ultrasound: 43±43; CI, 16 to 69), or Rankin (tPA only: 3±2; CI, 2 to 3 versus tPA plus ultrasound: 4±2; CI, 3 to 3). The neurological outcome was not influenced by the time to initiation of treatment or by the severity of hemorrhagic complications (no hemorrhage versus HT versus PH versus SAH).

Initial MRA in 9 of 12 patients treated with tPA only and 6 of 14 patients treated with combined tPA plus ultrasound revealed an occluded M1 segment. Five of 14 patients in the combined tPA plus ultrasound group had M2 segment occlusions on the initial MRA (Figure 2) as compared with none of 12 in the tPA only group. One of 12 patients had M3 occlusion entered the recombinant tPA only treatment branch. Five patients had occlusions of the ICA on initial MRA (2 in the tPA only and 3 in the combined treatment group). Partial or complete recanalization occurred in 6 of 12 patients treated with tPA only, but only in 4 of 14 patients treated with the combination of ultrasound plus tPA ($P=0.2629$). Recanalization was associated with the time to initiation of treatment in the tPA plus ultrasound group, as well as in the control group (Table 3).

Discussion

The TRUMBI trial was stopped prematurely because of an unexpected high rate of bleeding in patients treated with tPA plus low-frequency ultrasound. The bleeding rate was higher than published in studies of tPA treatment alone and higher than seen in the TRUMBI patients treated with tPA only. Parenchymal hemorrhages occurred exclusively in the combined treatment target group. We also found a high rate of atypical hemorrhages in the tPA plus ultrasound group either in the subarachnoid or in the ventricular space outside the brain or at remote locations distant to the infarct core. As expected, occurrence of bleedings was associated with time to initiation of treatment. This effect, however, was equally distributed between the treatment groups.

To our knowledge, this is the first acute stroke trial that used MRI as the only imaging modality for inclusion and with serial MR follow-up in 7 modalities (T1, T2, FLAIR, DWI, ADC, perfusion-weighted imaging, T2*, and time-of-flight MRA). Pretreatment exclusion of hemorrhagic stroke as well as identification of secondary bleeding (directly after treatment or at day 1) was based on T2* and FLAIR imaging. Recent studies have compared MRI to CT as a pretreatment tool to rule out hemorrhagic stroke.[15] MRI has been used to identify ischemic lesions[16] and vascular occlusion before thrombolysis but no study has used MRI only to rule out cerebral hemorrhage before treatment or to detect secondary hemorrhage after thrombolysis. Correlation to later CT imaging (day 1 to 3) in our small study

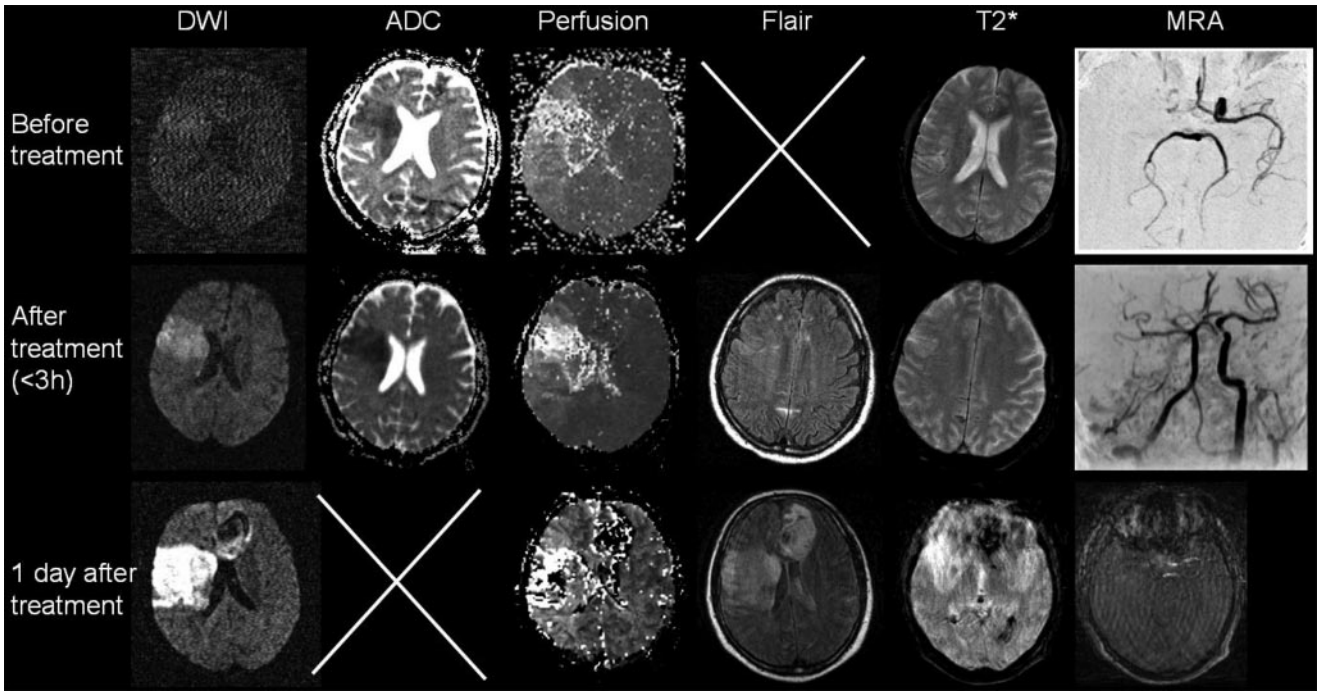

Figure 3. A 68-year-old patient with an acute left-side hemiparesis (NIH 13). Treatment (tPA plus ultrasound) started 160 minutes after symptom onset. Pretreatment MRI (upper row) showed left hemispheric hypoperfusion extending the DWI deficit. T2* imaging revealed no signs of bleeding. MRA showed occlusion of the ICA and T-junction. Posttreatment MRI (middle row) displayed some SAH but no PH in T2* and FLAIR measures. MRA showed no recanalization. Posttreatment NIH was 14. Follow-up MRI 18 hours after symptom onset (lower row) was conducted earlier because of vomiting and somnolence. DWI and FLAIR imaging showed middle cerebral artery infarction on the right side. T2* and FLAIR measures also showed extended SAH particularly in the frontal area on the right side and a significant PH in the left frontal region distant from any ischemia. NIH was 18. The patient deteriorated the next day, showing loss of consciousness and progressing signs of brain pressure despite maximal treatment, and died on day 3.

showed a higher number of hemorrhages detected with MRI but not with CT. Although use of MRI may account for the overall high rate of bleeding complications, the group differences suggest that ultrasound increased the risk of hemorrhage. Because tPA treatment was already approved when the trial started, there was no group without tPA treatment and ultrasound treatment alone. Therefore, the question of whether the combination of ultrasound and full-dose tPA or if the specific ultrasound used here by itself causes bleeding remains unclear.

In this small study, symptomatic hemorrhages showed a higher frequency in the target group. Four of 5 patients with symptomatic hemorrhages improved within the 3-month observation period to at least the pretreatment level. The remaining patient, however, died from secondary hemorrhage (Figure 3). There were 2 other deaths in the target group that are probably unrelated to the treatment. One patient died from myocardial infarction on day 3 that retrospectively likely occurred before treatment, and 1 patient died from pulmonary embolism ≈1 month after treatment. Neurological and functional scores after 3 months did not differ between the treatment groups significantly.

Our human results are in contrast to experimental animal studies[2,17] using a wide range of ultrasound, including ultrasound emission protocols that mirror our device. Our results are in stark contrast to the clinical observations and studies that used diagnostic available ultrasound in the range of 2 MHz.[12,18–20] Experimental studies indicated a better skull penetration and a higher effect of ultrasound in accelerating tPA–mediated thrombolysis with low frequency. An increased bleeding rate had not been reported in any of the experimental animal studies. The potential causes for hemorrhage in patients treated with low-frequency ultrasound and thrombolysis include differences in skull geometry between the small animals studied and the humans. It may be speculated that reverberations of the long wavelength ultrasound occurred inside the head, leading to "hot spots" of ultrasound energy. Low-frequency ultrasound may cause mechanical distortion of the human brain microvessels. Other differences in brain and vessel anatomy or differences in coagulation conditions may also play a role. Additionally all experimental studies had been conducted on young animals without any prestroke brain pathology.

Most other clinical studies using higher frequencies (2 MHz) did not find any increase in secondary hemorrhage.[12,18,19] Only one recent, small, single-center trial using duplex sonography for sonification also reported cerebral hemorrhages in the ultrasound group.[20] In that study, 2 of 11 patients had symptomatic hemorrhage and 2 of 11 patients had asymptomatic hemorrhage in the combined ultrasound plus tPA treatment group. This compared with 0 symptomatic and 1 asymptomatic hemorrhage in the 14 patients in the tPA only group. In this study and in TRUMBI, a larger transcranial ultrasound probe was used as compared with a 1-cm probe used in the combined lysis of thrombus in brain ischemia using transcranial ultrasound and systemic tPA trial[12] (CLOTBUST). It is likely that a greater region of brain was exposed to ultrasound in the 2 studies showing increased hemorrhage. The small probe used for con-

ventional diagnostic transcranial Doppler is purposefully targeted at the base of the brain and minimizes delivery of ultrasound energy to the brain itself.

In contrast to the diagnostic devices, we used low-frequency ultrasound (300 kHz versus 2 MHz). Variables that were previously associated with safety of ultrasound for biological tissue are well-known[21] and our device was within those limits. We chose low frequency to overcome the bone window limitation and to widen the sonification field to spare long-lasting targeting procedures that require highly skilled ultrasonographers. The underlying mechanism causing the high rate of hemorrhages is not clear. The high rates of SAH particularly lead to speculation of some mechanical action from the ultrasound disruption of small vessels in the subarachnoid space. Other biological effects that may play a role are vasodilatation from ultrasound[22,23] and opening of the blood–brain barrier.[17,24]

There are a few clinical observations that show effectiveness[12,19,20] with higher numbers of patients showing fast remission of symptoms. Our study did not show a positive effect on recanalization or clinical/functional outcome.

In conclusion, low-frequency ultrasound insonation in acute stroke can increase hemorrhagic rates even in the energy ranges considered to be safe. Whether this depends on the combination with tPA treatment or is an ultrasound-intrinsic biological effect is unclear and needs further investigation. This is particularly disturbing because small animal experiments failed to show this effect or did not adequately address the biophysics of the issue. We found increased hemorrhage when low-frequency ultrasound was combined with tPA, but given previous similar report with a higher frequency transcranial device, careful investigation of hemorrhage rate after combined thrombolysis with brain exposure to any ultrasound device is warranted.

Appendix

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