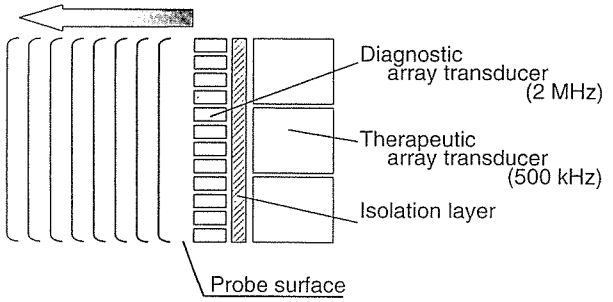

Fig. 2. Structure of T/D compound probe.

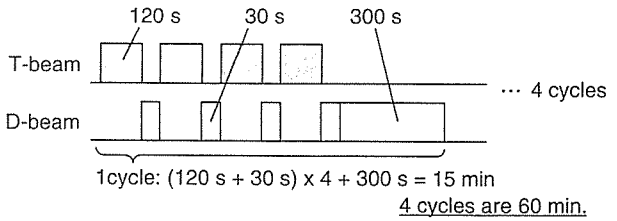

Fig. 3. Ultrasonication conditions.

array transducers laminated in one vessel. The T-beam frequency is 500 kHz and the D-beam frequency is 2 MHz, and the transducers are in different frequency bands. It is good for electroacoustic conversion efficiency that the therapeutic and diagnostic transducers have discrete apertures. However, the probe becomes too large what is too large to irradiate ultrasound beams to the brain (e.g., middle cerebral artery) through the cranial bone, the acoustic (echo) windows of which are very limited. Even the temporal bone window, one of the largest acoustic windows, is so narrow (the vertical and horizontal lengths are approximately 30 and 50 mm respectively) that it is irrelevant to search the affected area and irradiate T- and D-beams to it, if they have separate apertures.

Because it is necessary to transmit both T- and D-beams effectively through narrow bone windows, both T- and D-beam array transducers are laminated. The D-beam array is formed on the T-beam array backwards and emits ultrasound directly through the acoustic lens that is the surface of the probe, as shown in Fig. 2. Owing to the structure, the both arrays can use a narrow temporal bone window effectively and do therapy and diagnosis quasi-simultaneously. Because both transducers' structures are of the array type, T-beams can be irradiated to any particular part on the diagnostic image plane in the brain by, for example, B mode and TCCFI. Moreover, if the therapeutic and diagnostic array transducers are laminated directly, both T- and D-beams can interfere with each other. Therefore, this probe has an isolation layer between them.

This probe can obtain an ultrasound image of commercial-probe quality using its D-beam. The T-beam can be deflected 45° from the vertical line and the focal length of the beam can be controlled between 20 and 150 mm from the surface of the probe. Therefore, we can search the affected area on the ultrasound B mode image and the affected area can be targeted with the T-beam. Moreover, during therapy, we can observe blood flow and determine its recanalization. The T-beam width (FWHM) is 7.5 mm along the elevational axis and 4.0 mm along the lateral axis at a 28 mm focal length.[10]

2.3 *Ultrasound exposure protocol*

The experimental setup is designed to expose blood clots to both T-beam (500 kHz) and D-beam (2 MHz) ultrasounds by the intermittent exposure method assumed for clinical application. This system's ultrasound exposure repeats for four cycles in a therapeutic period; each cycle consists of periods of the T-beam being applied for 120 s and the D-beam being applied for 30 s repeated four times followed by the D-beam being applied for 5 min. One cycle lasts 15 min and four cycles last 60 min. Ultrasound is emitted as shown in Fig. 3 in which the duty cycle is 53% for the T-beam. The D-beam is in the TCCFI mode, and the T-beam is cw to enhance the thrombolysis effect of t-PA.

2.4 *Thrombus formation model*

Two different types of clots were applied to the following experiments. One is made from fresh human blood applied to the recanalization experiment in which the clot is placed in the narrow part of the syringe to stop the flow of a saline solution.[11] The other is made from horse blood applied to the fundamental dissolution rate experiment.

In the recanalization experiment, a clot is made from 1.25 ml of freshly drawn blood from healthy subjects. The clot is left still for 40 min and centrifuged on a centrifugal separator for 5 min. In the dissolution rate experiment, a clot is made from 0.8 ml of wet preserved horse blood, to which 0.1 ml of $CaCl_2$ (25 mM/ml) and 0.1 ml of thrombin (5 mM/ml) are added. The solution is left for 15 min in an incubator that is maintained at 37 °C and centrifuged on a centrifugal separator for 5 min. In each experiment, we use four clots per handling.

2.5 *Recanalization experimental system*

Figure 4(a) shows the thrombo embolism model. An artificial clot is placed in a syringe filled with saline and t-PA, which is made to a density of 358 IU/ml, as in clinical use. The internal diameter (ID) of the syringe is 9 mm and ID at the arctia (constricted portion of the syringe) is 2.5 mm, where the clot is stopped.

Figure 4(b) shows the experimental setup for ultrasound irradiation. The thrombo embolization model standing in the water bath is maintained at 37 °C with a heater. The experiments are performed under two types of condition in this bath; the clot is exposed to ultrasound 0.05 W/cm^2 in intensity emitted by the T/D compound probe in one condition, and not exposed to ultrasound in the other. The former is called the US+ group and the latter is called the US− (t-PA-only) group. (US+ group refers t-PA solution with ultrasonication and t-PA-only group refers t-PA solution without ultrasonication hereafter.) The T/D compound probe and sonication syringe are covered with an ultrasound absorber so as to not reflect ultrasound beams by the wall of the water bath and not expose the US− group. In one experiment, there are one US+ syringe and three US− syringes. The Clot dissolves slowly, and when it becomes sufficiently small to go through the arctia, the whole clot falls down to the bath along with the water above the clot. We define the time from the injection of t-PA solution to the

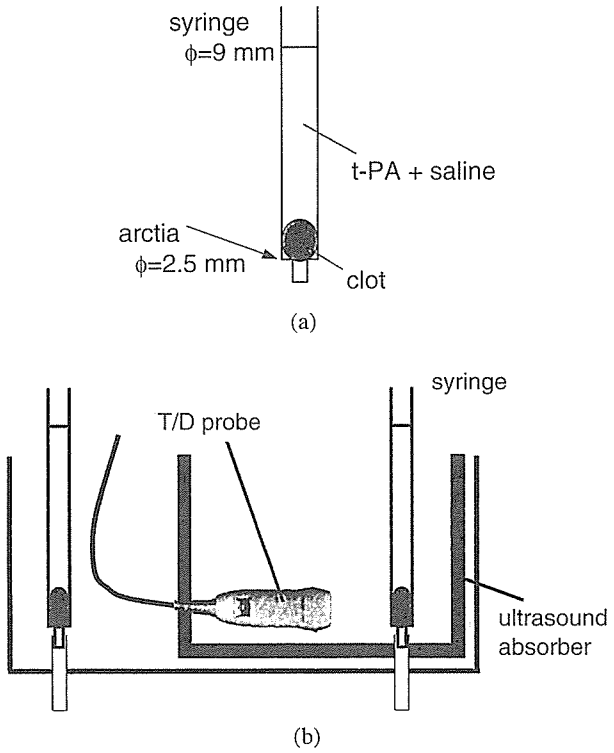

Fig. 4. Experimental setup of ultrasound irradiation. (a) Thrombo embolization model. (b) Setting in water bath.

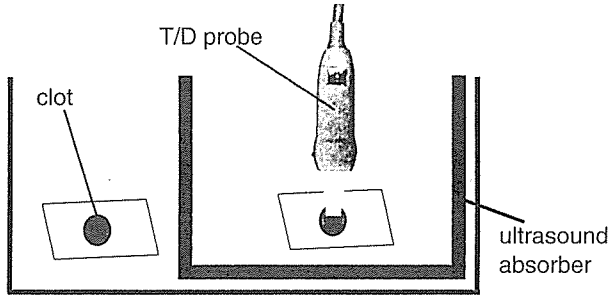

Fig. 5. Experimental setup of ultrasound irradiation.

instant that the clot falls down through the arctia as the recanalization time, and compare it between the US+ group ($n = 13$) and the US− group ($n = 39$).

2.6 *Dissolution rate experiment*

The artificial blood clot was placed in a polyethylene bag with a solution of saline and t-PA, the density of which was 358 IU/ml, the same as that in clinical use. One clot was ultrasonicated at a 0.05 W/cm^2 intensity at the clot using the T/D compound probe (US+, $n = 6$) and the others (US−, $n = 17$) were not sonicated. They were all incubated in 37 °C maintained water with a heater (Fig. 5). To measure the dissolution rate, their weights were compared after 60 min in this system before the sonication.

3. Results

3.1 *Result of recanalization experiment*

The relationship of recanalization rate to duration is shown in Fig. 6. The recanalization rates 60 min after the administrations of t-PA were 92.3% for the US+ group, and

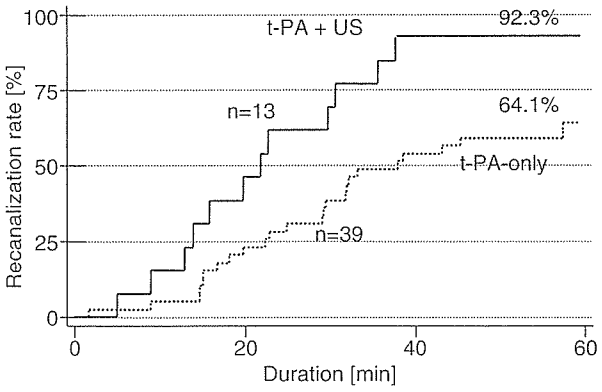

Fig. 6. Recanalization rate vs duration from US exposure. Comparing t-PA + US with t-PA-only.

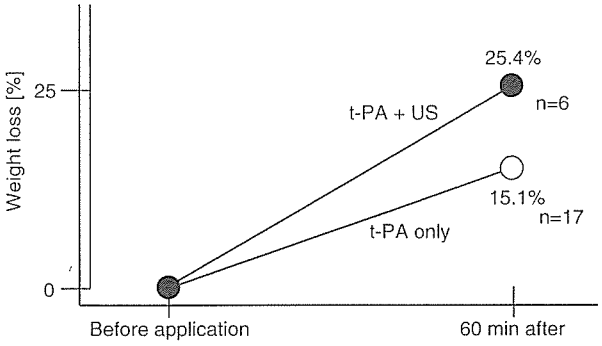

Fig. 7. Weight decrease of clots, before vs 60 min after sonication *in vitro*. Comparing t-PA + US with t-PA-only.

64.1% for the US− group. The average recanalization times were 21.4 min for the US+ group, and 27.2 min for the US− group. Elapsed time for recanalization has a significant difference between the two groups ($p < 0.0062$).

3.2 *Result of dissolution rate experiment*

Figure 7 shows the average loss of weight 1 h after the administration of t-PA solution. The weight loss at 60 min is 25% for the US+ group and 15% for the US− group. The dissolution rate of the US+ group is 1.7 times higher than that of the US− group. Although these results are not significantly difference, they suggest that the ultrasound from the compound transducer system accelerates thrombolysis, as in the literature.

4. Discussion and Conclusions

In this study, it is confirmed that the average recanalization rates were 92.3% for the US+ group, and 64.1% for the US− group, and the weight losses were 25.4% for the US+ group and 15.1% for the US− group 60 min after the administration of t-PA. We confirmed that our newly developed system with the T/D compound probe and intermittent irradiation using a low-frequency (500 kHz) low-intensity ($I_{SPTA} = 0.05$ W/cm^2) T-beam enhances the effect of the thrombolytic agent t-PA.

We assume that the maximum ultrasound intensity in the clinical use is 0.72 W/cm^2 (I_{SPTA}), which weakens to 1/5–1/6 this value owing to the attenuation caused by absorption and scattering in the cranial bone. Therefore, the reduced

ultrasound intensity is approximately $0.12\,W/cm^2$ at the affected area in the brain. In this experiment, a clot was irradiated at an intensity of only $0.05\,W/cm^2$, which is much smaller than the maximum reduced intensity after having passed through the bone. This fact, in addition to the above-mentioned results, suggests that the system has a high thrombolysis performance even at safe intensities with sufficient safety margins. Using the developed system, further clinical studies will be conducted on *in vivo* experiments of model blood vessels weakened owing to old age.

In the future, the relationship between ultrasonic intensity and recanalization rate will be made clear, for clinical use. The study of Daffertshofer *et al.* indicates that low-frequency ultrasound may have adverse biological effects[9] because of the influence of cavitation or another reasons. Therefore, to reduce the possibility of cavitation that may be caused by standing waves[12] and that might hurt the brain tissue, we will verify safe exposure conditions for not only continuous waves but also burst waves and fast small-angle deflection experiments hereafter.

Acknowledgement

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