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Timing of Microbubble-Enhanced Sonothrombolysis Strongly Predicts Intracranial Hemorrhage in Acute Ischemic Stroke

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Background: Although ultrasound-activated microbubbles (MB) accelerates clot lysis, MB activation has shown to promote blood barrier disruption and hemorrhagic transformation in animal models. We conducted a case-control study aimed to investigate the risk of HT after MB-enhanced sonothrombolysis in acute stroke.

Patients and Methods: We prospectively evaluated 188 patients with acute stroke related to MCA occlusion and treated with i.v. tPA < 6 hours of stroke onset. Patients received continuous 2-hour TCD monitoring plus 3 doses of 2.5 g of galactose-based MBs given at 2, 20, and 40 minutes after tPA bolus (MB group). These patients were compared with 98 historical stroke patients treated with tPA plus 2-hour TCD monitoring (control group). Both timing and degree of recanalization during first 12 hours of tPA bolus were recorded. Presence and extend of HT on 24-h CT was blinded assessed as HI1, HI2, PH1 and PH2.

Results: Median baseline NIHSS was 17. Age, baseline NIHSS, clot location, early CT findings, stroke subtype and time to treatment were similar between MB and control group. Recanalization rates at 1h (32.2% vs 21%), 2h (50.0% vs 36.7%), 6h (63.8%/44.5%) and 12h (74.3%/56.2%) were significantly higher in the MB compared to the control group ($p < 0.05$). MB administration was significantly associated with an increased risk of HI1-H2 (21% vs 12%, $p = 0.026$ OR 5.8 95% IC 2.1-65), and higher degree of clinical improvement at 24h (54.9% / 31.1%, $p = 0.004$). PH1-PH2 (3.3% vs 3.8%; $p = 0.8$) and symptomatic ICH rates (2.9% / 2.1%, $p = 0.580$) were comparable in both groups. Moreover, the extend of bleeding after MB-enhanced sonothrombolysis was linked to the time-to-reperfusion. Early (<6h) recanalization independently predicted HI in the MB group (OR 6.3 95% IC 2.3-56) but not in the control group. Delayed (>6h) or no recanalization (>6h) was significantly associated with PH1-PH2 in both MB ($p = 0.024$) and control group ($p = 0.045$), respectively.

Conclusion: The extend of bleeding after MB-enhanced sonothrombolysis is linked to the time-to-reperfusion. MB administration is associated with early recanalization and high rate of HI1-HI2, but it does not increase the risk of symptomatic ICH.

04

Sonothrombolysis Acceleration by Bubble Liposomes in vitro

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Purpose: The rapid acceleration of sonothrombolysis by combined use of 500kHz middle-frequency ultrasound (US), recombinant tissue-plasminogen activator (rt-PA), and bubble liposomes (BLs) was verified in vitro by the reduction of clot weight in 60 seconds.

Methods: A fibrin clot was formed by adding thrombin to bovine plasma. It was enclosed in a pressurized container, the pressure and temperature of which were maintained at 150mmHg and 37°C, respectively. Ultrasonic conditions were set at a continuous waveform, a frequency of 500kHz, an intensity of 0.7W/cm², and a sonication time of 60s. We derived the rate of reduction in clot weight from the decreased clot weight and the weight before sonication. We compared the rate of reduction in 8 groups combining physiological saline, rt-PA, BLs, and US. 10 clots were assigned to each group.

Results: There was no significant difference in the mean clot weight of each group. Only the rt-PA+US+BLs group showed a significantly accelerated thrombolytic effect compared with any other group or with any combination of two factors in the 60-s period ($0.001 < P < 0.027$).

Conclusion: It is anticipated that BLs have great potential to accelerate rapidly the sonothrombolytic effect of rt-PA with middle-frequency, 500-kHz, continuous-wave ultrasound.

SESSION 2

05

Usefulness of an Ultrasound Perfusional Approach and Derived Maps for Predicting the Prognosis of MCA Occlusion and T-Type Occlusion Stroke Patients

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It has well known from the recent years that the diagnosis of vessel occlusion site in the acute phase of stroke, as made by TCCS, has a prognostical role. In the population admitted

to the Stroke Unit of Reggio Emilia from 1 January 2007 to the 29 February 2008, 73 patients with MCA or T occlusion were evaluated within six hours from the onset of acute stroke symptoms were selected. All patients (age 74.3 ± 7.6 years, 56% men) underwent to supraaortic trunks sonography and TCCS with contrast medium injection for perfusional and large vessels examination. Direct visual and off-line measurement of perfusional data with tridimensional mapping were performed for all patients. MCA and T occlusion was diagnosed according to the literature criteria. Forty patients were treated by intravenous and six by intraarterial thrombolysis; the remaining patients underwent to sonothrombolysis. During Stroke Unit staying eight patients died by intracranial hypertension and four for caused not related to stroke. Only two patients with T occlusion had a complete recanalization at one hour from the diagnosis and then they had a good prognosis (mRS 0-2). Perfusional quantitative parameters and visual grading of the maps were more markedly impaired in the affected hemisphere in all patients who died than in survivors and no recanalization was found in the former.

TCCS in the acute phase can give main prognostical informations and the perfusional approach can further improve the accuracy of diagnostic and prognostic evaluation.

06

Safety Evaluation of Newly Developed Transcranial Targeting Low-Frequency Ultrasonic Thrombolysis System (TCT-LoFUT) at 490kHz for Macaca Monkey's Brain

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Background and Purpose: We applied a developed TCT-LoFUT for AIS at 490kHz continuous waveform (CW) US to the healthy monkeys' brain via sonication of the ipsilateral MCA. **Methods:** Nine young cynomolgus monkeys were assigned

to three groups, consisting of three monkeys each: a control group without sonication (Control), the second group of monkeys raised for one day after sonication (U1) and the third group raised for seven days after sonication (U7). Two aged rhesus monkeys were sonicated under transvenous injection of alteplase (0.9mg/kg), and were raised for seven days (R7). A switching circuit controlled the operation of 1) the therapeutic US beam (T-beam) generator for thrombolysis at 490kHz, 0.72W/cm² and 2) the diagnostic TC-CFI US beam (D-beam) at 2.5MHz, 0.20 W/cm². One set of 120-second T-beam active state and 30-second D-beam active state were repeated four times. After that, the T-beam was stopped and the D-beam was activated for 5 minutes. This 15-minute protocol was repeated four times continually for 60 minutes. R7 group was examined with the MR imaging one day after sonication. The brains were immediately removed and the thirteen immunohistochemical staining were performed in all cases. Using three pieces of monkey's skull, 490kHz CW-US was sonicated and measured transcranial intensity.

Results: The U1, U7 and R7 groups showed no tissue damage microscopically and there was no hemorrhagic transformation at MR imaging in R7 group. Measured transcranial US intensity rate was $47 \pm 12\%$. The intracranial MI was 0.14.

Conclusions: The TCT-LoFUT did not cause any tissue damage in primate brain.

07

Asymptomatic Carotid Emboli Study (HACES): Baseline Data

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Background: Better methods of identifying which patients with asymptomatic carotid stenosis (ACS) develop stroke are required to improve risk-benefit ratios for endarterectomy. A promising method is detection of asymptomatic embolic signals (ES) using transcranial Doppler (TCD). ES predict stroke risk in symptomatic carotid stenosis but data in ACS is conflicting.

Method: ACES is a prospective study in ACS $\geq 70\%$. Two 1 hour ipsilateral MCA TCD recordings are performed at baseline, and a single hour recording at 6, 12, and 18 months. Follow up is 2 years. All recordings are centrally analysed. The primary endpoint is: Do ES on either of 2 baseline recordings predict ipsilateral TIA and stroke over 2 years? Secondary endpoint: Do ES on a single hour recording predict risk over any subsequent 6 month period?

Results: Recruitment completed October 2007 with 482 patients. Baseline demographics were: mean age 71.49 years; hypertension 79%; smoking: current 14.52%, ex 46.47%;