SYNOPSIS

Title of Study: Sonothrombolysis potentiated by microbubbles as a novel treatment of acute ischemic stroke: a prospective randomized pilot study **EudraCT number:** 2012-000512-29 **Sponsor protocol number:** IIBSP-SPM-2011-63
Investigators: Joan Martí-Fàbregas
Study centre(s): Hospital de la Santa Creu i Sant Pau
Publication (reference): none
Studied period (years): Date of first enrolment: Sep 15 2012 Date of last completed: Feb 6 2015
Phase of development: Therapeutic exploratory trial (Phase II)
Objectives: To evaluate the safety and efficacy of combination therapy: Intravenous recombinant tissue plasminogen activator (rtPA) + Ultrasound (US) + Microbubbles (MB) vs. standard treatment with rtPA in a randomized study of patients with acute ischemic stroke. The synergy of the effect of US and MB on sonothrombolysis (ST) has been previously proposed in a case-control study. However, the acceleration of ST with the use of MB is still the subject of research. There are no randomized studies in the literature looking at the benefits of combination therapy. Therefore, we want to investigate the safety and efficacy of the diagnostic contrast medium in the form of sulfur hexafluoride MB, in the recanalization of the middle cerebral artery (MCA) during the application of systemic thrombolysis and continuous monitoring of Transcranial Doppler (TCD) with a pulsed 2Hz waveform.
Methodology: This was a double blind, prospective, randomized, phase II trial. Acute ischemic stroke patients with documented arterial occlusion ofthe middle cerebral artery and treatable within 4,5 hours, were randomized to one of two arms: combined treatment with sulphur hexafluoride- filled MB, transcranial Doppler (TCD) and systemic rtPA (MB group) compared to systemic rtPA alone with brief TCD vessel diagnostic assessments (control group). Study endpoints: recanalization rate (according to the TIBI grading system), hemorrhagic transformation rate, clinical improvement (NIHSS at 6- 24 h) and mortality (24 h and 3 months), 3 months clinical outcome (favourable outcome defined as Rankin scale score 0-2).
Number of patients (planned and analysed): We planned to randomize 120 patients (treatment with 60 rtPA vs. 60 with rtPA + UA + MB). Finally, twenty-four patients were included out of a total of 144 patients who received reperfusion treatments in the study period.
Diagnosis and main criteria for inclusion: The primary inclusion criteria: 1) Patients with acute ischemic stroke (<4.5 hours) of the territory of the middle cerebral artery (MCA), either due to clinical suspicion or by CT imaging, with a neurological deficit quantifiable with the National Institute of Health Stroke Scale (NIHSS), which in the opinion of the responsible physicians requires and meets standard treatment criteria with intravenous rtPA at a dose of 0.9 mg / kg, in accordance with the criteria authorized by the Ministry of Health. 2) A documented occlusion of the MCA by TCD and / or CT angiogram (segments M1 and M2). 3) Signature of consent to participate in the trial by the patient or her legal representative.

Test product product, dose and mode of administration:
Experimental Drug: Sulfur Hexafluoride (sonovue), Powder and solvent for injectable dispersion.
SonoVue is a kit that includes: 1 vial containing 25 mg of lyophilized powder, 1 pre-filled syringe with 5 ml of sodium chloride

Time since rtPA infusion	Dose	Route of administration
0'	5 ml	Slow intravenous bolus (1')
20'	5 ml	Slow intravenous bolus (1')
40'	5 ml	Slow intravenous bolus

Duration of treatment:
1 hour

Reference therapy, dose and mode of administration:
Control drug : Standard thrombolytic therapy

Criteria for evaluation:
Efficacy: The short term efficacy of treatment will be evaluated by recanalization rate at 6 hours control TCD (TIBI score) and at 24 hours by the NIHSS (a> 4 points improvement).
Safety: Serious adverse events should not exceed the reported rate in the EMA official Sonovue product information

Statistical methods:
Analysis is performed with SPSS software (SPSS Inc., Chicago, IL). For categorical variables, the chi-square test is used. For ordinal or quantitative variables, the non-parametric test (Mann - Whitney) is applied in two groups and basic descriptive statistical parameters (median, minimum and maximum) of both are provided. Finally, with respect to the quantitative variables that satisfy the assumptions of normal distribution and homogeneity of variance, a Student's t test is applied for two independent groups and the mean and standard deviation will be provided. A comparative analysis of the main variable is carried out. The primary endpoint is dichotomized (recanalization yes / no) and its proportion between the 2 therapeutic groups will be compared with contingency tables ($\chi 2$ test and Fisher's exact test). All tests used a significance level of 5% ($\alpha = 0.05$), a two-tailed approach (two-tailed), and a power of 80%. For the analysis, the statistical package PASW (V.18.0) will be used.

Summary
Results:
A total of 24 patients were randomized (MB 11, controls 13). Complete recanalization rates (MB 54,5 % vs controls 46,2%), were similar in both groups. No differences were found in terms of clinical improvement at 24 hours (MB 9% vs controls 9%) and 90 days (MB 27,3% vs controls 30,8%) nor in terms of symptomatic (MB 0% vs controls 7,7%) and asymptomatic (MB 27,3% vs controls 38,5%) intracranial bleeding. There were 2 deaths in each group, none related to the treatment under investigation.
Conclusion
Sonothrombolysis potentiated by microbubbles is safe in our small pilot study. Efficacy on recanalization rates and outcomes needs to be confirmed in larger randomized series.

Date of report
Feb 01, 2016