SONOTHROMBOLYSIS ENHANCED WITH MICROBUBBLES IN ACUTE ISCHEMIC STROKE: A RANDOMIZED PILOT TRIAL

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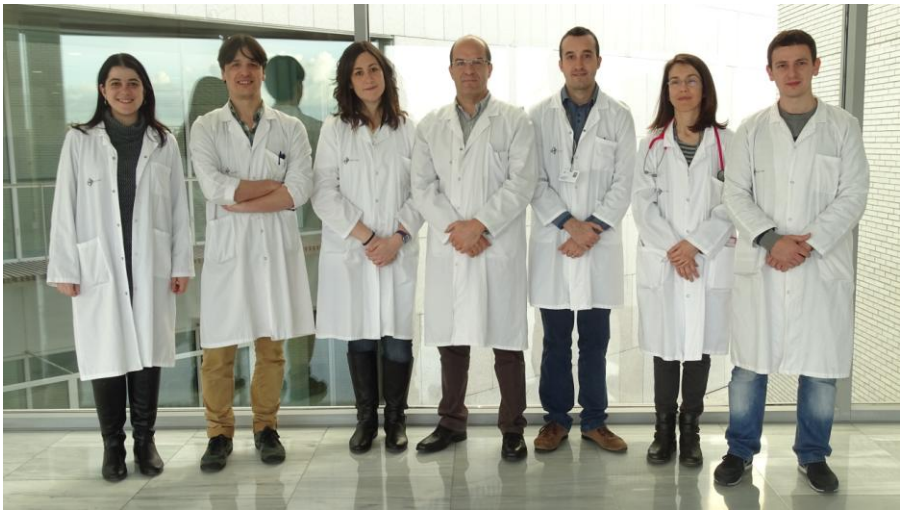

1. Summary

Background

Acute ischemic stroke is due to the sudden occlusion of an intracranial artery, causing ischemia in the parenchyma irrigated by this artery. Intravenous thrombolysis with rt-PA (recombinant tissue plasminogen activator) administered within the first 4.5 hours was, up to 1 year ago, the only approved treatment for acute ischemic stroke. Early administration of the drug increases the chance of achieving the recanalization of the arterial occlusion and as a consequence the early reperfusion of salvageable ischemic tissue and clinical improvement. However, in patients with a proximal intracranial large-artery occlusion, rt-PA is often unable to achieve this recanalization quickly and completely. It is estimated that rt-PA recanalization is obtained only in 20-46% of patients with occlusion of the TICA (intracranial internal carotid artery) or M1 (first segment of the middle cerebral artery -MCA-).

Thus, we need further therapeutic strategies to increase the rate of recanalization in these proximal occlusions. The two methods that have been evaluated are sonothrombolysis (ST, thrombolysis with rt-PA enhanced with the application of ultrasound at the point of occlusion) and endovascular treatments (mechanical thrombectomy with stent retrievers in selected patients). This latter treatment is applied in patients who already started treatment with rt-PA and its effectiveness has been demonstrated in trials published in 2015.

Several studies and meta-analyses showed that sonothrombolysis (ST) increases the rate of recanalization provided by rt-PA. Its effect is accomplished by the ultrasound-induced increase in the interaction of rt-PA and fibrin thrombus. ST can easily be administered at the patient's bedside and it appears to be a low-risk treatment. Nonrandomized studies suggested that ST combined with the simultaneous administration of sulfur hexafluoride microbubbles (MB) induces a further increase in the chance of recanalization compared with ST, and therefore leads to more rapid and complete recanalization. Microbubbles (MB) are small gas spheres with specific acoustic properties, which in contact with US oscillate nonlinearly. The application of high-pressure acoustic US implies continuous energy absorption until the bubble bursts, releasing the stored energy. The destruction of the MB with US can accelerate the effect of thrombus dissolution by the US. However, there is no randomized study

available to demonstrate the efficacy and safety of ST combined with MB (ST + MB) compared with thrombolysis with rt-PA alone. Therefore, our objective was to conduct a pilot randomized phase II clinical trial. With this study we hope to evaluate the safety and efficacy trends of ST + MB.

Methods

Type of study. Double-blind, randomized, parallel assignment, Phase II study, registered in *www.clinicaltrials.gov* with the number NCT016784495. The study was approved by the Ethics Committee of the Hospital de la Santa Creu i Sant Pau, and all patients or their legal representatives signed an informed consent to participate in the study. Patient recruitment started in September 2012 and ended in June 2015. The publication of clinical trials of mechanical thrombectomy made us consider that it would be unethical to continue enrolling patients.

Inclusion and exclusion criteria. We included consecutive patients with: 1) criteria for standard treatment with intravenous rt-PA; 2) MCA occlusion (segments M1 and M2); 3) signed the consent to participate. Patients were excluded: 1) NIHSS score> 25; 2) rapid spontaneous improvement; 3) those receiving endovascular treatment; 4) previous Rankin scale score >1; 5) any contraindication for intravenous thrombolysis with rt-PA and 6) temporal-bone poor acoustic window.

Scales. The severity of neurological deficit was measured by the NIHSS scale at admission and at 1, 6, 12 and 24 hours after the onset of symptoms. The functional outcome at 3 months was calculated by the Rankin scale score. The degree of occlusion was assessed by transcranial Doppler (TCD) and was defined according to the TIBI score (Thrombolysis in Brain Ischemia) on admission, and at 1, 6 and 24 hours after initiation of treatment. *Bleeding complications*. Symptomatic hemorrhage was defined as a PH-type hemorrhage that appears within the first 36 hours after treatment, and causes an increase of 4 or more points on the NIHSS score.

Primary variable: Rate of total and partial recanalization (according to TIBI criteria). *Secondary variables*: Functional prognosis at 3 months; rate of symptomatic intracerebral hemorrhage; clinical improvement (NIHSS at 24 hours) and mortality. *Randomization and blinding.* Patients were randomly assigned to one of the two therapeutic arms: ST + MB + rt-PA or rt-PA. Patients were unaware of the treatment

group to which they were assigned. The neurologist who performed the treatment obviously knew the details of the treatment, but the neurologist who evaluated patients at 3 months was blinded to clinical data.

Technique. ST is applied by administering transcranial ultrasound continuously at the depth where the trunk of the ACM is expected to be (approximately 50 mm deep) for 1 hour at a frequency of 2 MHz. Patients in the group of ST + MB were treated with a combination of continuous TCD for 1 hour, and three boluses of 5ml (8 µl/ml) of sulfur hexafluoride MB (Sonovue®) administered at 2, 20 and 40 minutes after the onset of infusion.

Statistics. The principal variable is categorical (recanalization yes / no) and comparison between treatment groups was done with Chi-square test.

2. Results

During the study period a total of 24 patients were included; 13 cases were treated with intravenous rt-PA and 11 cases with the combined study treatment (ST + MB + rt-PA). The median age of patients was 77 years and 54.1% were men. The median baseline NIHSS score on the scale was 17.5. A total of 64.7% showed early signs in the baseline cranial CT with a median baseline ASPECTS scale score of 8. MCA occlusion was demonstrated in all patients by CT angiography or Doppler / Duplex. Since the beginning of the study, in addition to the 24 patients enrolled, 120 patients were evaluated who have not been included for various reasons.

Overall results for group: Demographic data, risk factors, and clinical and radiological characteristics of both groups showed that age, NIHSS score on admission, Rankin Scale score after the stroke, the findings of ischemia early on CT, stroke subtype and time to treatment were equivalent in both groups. The median score on the NIHSS at admission was 18 (interquartile range 7-22) in the intravenous treatment group and 17 (IQ range 10-21) in the combined treatment group.

Results of safety and efficacy. Complete recanalization rates were similar in both groups at one hour (ST + 27.4% MB 38.5% vs controls), at 6 hours (ST + 54.5% MB

46.2% vs controls) and 24 hours (ST + MB 54.5% vs 61% controls) (p = ns). There were no differences regarding clinical improvement at 24 hours (ST + MB 9% vs controls 9%) and 3 months (ST + MB 27.3% vs controls 30.8%) or in relation to the occurrence of symptomatic intracerebral hemorrhage (ST + MB 0% vs controls 7.7%) and asymptomatic bleedings (ST + MB 27.3% vs 38.5% controls), or mortality (2 in each group).

3. Relevance and possible implications

Total or partial recanalization is an important predictor of good prognosis in patients with acute ischemic stroke treated with reperfusion techniques, either by intravenous or rescue thrombectomy. However, only 15-30% of proximal occlusions can be recanalized by rt-PA. Therefore a method for enhancing the capacity of recanalization is needed. Despite the efficacy of mechanical thrombectomy, the logistic for this treatment is complicated and its cost is high: the treatment is only available in some specialized centers and treatment criteria are very restricted. For all of the above, there are still reasons for seeking the enhancement of systemic thrombolysis with rt-PA, and this is the purpose of ST and ST enhanced with MB. Thus, we could indicate ST in patients who 1) receive intravenous thrombolysis, with the idea of reducing the number of patients who eventually will need thrombectomy; 2) When thrombectomy is not indicated (e.g. by age, NIHSS score / ASPECTS, an artery occlusion different from the M1 segment of the middle cerebral artery); 3) When thrombectomy is unavailable for being far from a tertiary center.

Of course the main drawback of the ST is the need of an expert examiner in this treatment, which is often not available 24/7 in tertiary centers. The unavailability of an expert examiner was one of the main causes of loss of patients to be enrolled in our study. Another drawback is that ST requires a temporal bone window of sufficient quality to allow the passage of ultrasound. Unfortunately, about 10% of the population does not have a good-quality temporal bone window.

However, in addition to these relatively restricted indications, treatment with ST must demonstrate that it is effective in randomized clinical trials and demonstrate that it is safe, especially in the risk of iatrogenic symptomatic intracerebral hemorrhage. A

meta-analysis of six randomized trials and 3 nonrandomized studies concluded that ST triples the likelihood of complete recanalization and could double the proportion of patients free of death or functional dependence, and without increasing the rate of symptomatic hemorrhage.

In addition, ST may be supplemented, as in our study, using MB. One of the theoretical risks associated with ST with or without MB is cerebral hemorrhage. Studies in experimental animals show that low frequency ultrasounds may promote disruption of the blood brain barrier and persistence of waves with consequent risk of hemorrhage. Pilot studies in humans at low frequency were also prematurely halted due to this complication (TRUMBI study). Prior to our study, another study (TUCSON) evaluated this enhancement of recanalization although nanobubbles were used, different therefore to those used in our study, and the study was stopped prematurely because several patients in the combined treatment group died of cerebral hemorrhage. Therefore, an important contribution of our study is to demonstrate that the risk of symptomatic brain bleeding complications is not increased in the group with ST + MB. Currently, there is an ongoing randomized Phase III study (CLOTBUSTER-ER) evaluating the efficacy and safety of the combination of rt-PA and ultrasound, but this study does not use MB.

Our study has limitations. The main limitation is the small number of patients included. However, the main contribution of the study is to provide randomized data on ST + MB, which are not available in the literature. In addition, we provide safety data from randomized patients and we have shown that the risk of symptomatic hemorrhage is not higher than the risk for patients receiving intravenous thrombolysis without further treatment. Our data do not establish an increased capacity of recanalization with combined therapy, because the patient sample (13 vs. 11) is clearly insufficient. Finally, the small number of patients does not allow subgroup analysis, which would have been very interesting. For example, to observe efficacy or safety depending on variables such as age, exact point of occlusion or etiology.

We believe that ST, despite the important alternative of endovascular treatment still has some indications as previously mentioned. It is necessary to design large-scale studies, randomized, placebo-controlled, with a sufficient number of patients with clinical end-point variables, to definitively determine whether the combination of ST +

MB shows a higher effectiveness and comparable safety than isolated thrombolysis. The dose, the number of times that MB need to be administered, the MB size and the moment to be administered should also be clarified. It is also possible to perform sonolysis, i.e. administration with or without ultrasound MB in patients not receiving thrombolytic.

Reperfusion therapy has revolutionized the management of patients with stroke and has made stroke a treatable medical emergency. ST+MB can become a tool in the treatment of the acute phase.

4. Generated literature

To be presented at the European Stroke Conference Organization (Barcelona 2016)
Sonothrombolysis potentiated by Microbubbles in acute ischemic stroke:
a prospective randomized pilot study

In preparation for publication in international journal:
Sonothrombolysis potentiated by Microbubbles in acute ischemic stroke:
a prospective randomized pilot study