Combining Statins with Tissue Plasminogen Activator Treatment After Experimental and Human Stroke: A Safety Study on Hemorrhagic Transformation

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SUMMARY

Aims: Statins may afford neuroprotection against ischemic injury, but it remains controversial whether combined treatment with tissue plasminogen activator (tPA) after stroke increases the risk of hemorrhagic transformation (HT), the major tPA-related complication. We evaluated the safety of combining statin with tPA administration during the acute phase of both experimental and human stroke. **Methods:** The occurrence and severity of HT, infarct volume, and neurological outcome were evaluated in spontaneous hypertensive rats (SHR) subjected to embolic middle cerebral arterial occlusion (MCAO), which received vehicle or simvastatin (20 mg/kg), 15 min after ischemia and tPA (9 mg/kg) 3 h after ischemia. Additionally, HT rate was evaluated in stroke patients who were treated with tPA (0.9 mg/kg) within 3 h after symptom onset, considering whether or not were under statins treatment when the stroke occurred. **Results:** In the experimental study, no differences in HT rates and severity were found between treatment groups, neither regarding mortality, neurological deficit, infarct volume, or metalloproteinases (MMPs) brain content. In the clinical study, HT rates and hemorrhage type were similar in stroke patients who were or not under statins treatment. **Conclusion:** This study consistently confirms that the use of statins does not increase HT rates and severity when is combined with tPA administration.

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Introduction

Stroke remains as a major cause of death and disability worldwide that contributes to the rising costs of health care [1]. Nowadays, the thrombolytic treatment with the tissue plasminogen activator (tPA) agent is the only existing therapy for the acute phase of stroke [2]. While high effectiveness on stroke outcome has been attributed to its use, tPA-treated stroke patients have a 10-fold higher risk of suffering an intracranial hemorrhage than untreated patients, which is fatal in about 3% of those patients [2–4]. Thus, the occurrence of hemorrhagic transformation (HT) after tPA treatment represents the main concern in the stroke acute therapy.

Animal studies have extensively shown that treatment with statin, including simvastatin, reduces the infarct volume and ameliorates the neurological deficit after experimental stroke [5,6]. These particular benefits in cerebral ischemia have not been attributed to the statin cholesterol lowering effects. Instead, pleiotropic effects, such as endothelial nitric oxide increase [7], reduction of oxidative stress [8], block of platelet

activation [9], and antiinflammatory actions [10], have been implicated in its protective role in stroke. These properties suggest statins as a safe and promising cotreatment for reperfusion therapies in the acute phase of stroke [11,12]. Statin-treatment advantages have also been shown in the clinical field. Statin pretreatment decreases the incidence of stroke patients at risk of cardiovascular disease [13,14]. Statin treatment before or early after an ischemic stroke triggers more favorable outcome in patients [15,16]. However, clinical and experimental studies investigating the risk of HT after combined tPA treatment with statin are scarce. Preclinical studies in animals have demonstrated statins safety when combined with tPA [17,18] although none of them have studied the effect of simvastatin and tPA combination as a posttreatment. Conversely, a recent clinical study has raised caution reporting an increased risk of HT among those tPA-treated patients that were under statins at the moment of having a stroke [19]. Therefore, special attention in evaluating the HT after coadministering statin and tPA is required before planning future clinical trials. In this study, we aimed to test both experimentally and clinically, whether the

combined treatment of statin plus tPA is a safe therapy in the acute stroke setting.

Methods

Experimental Study

All procedures were approved by the Animal Ethics Committee of the Vall d'Hebron Research Institute (02/09 CEEA) and were conducted in compliance with the Spanish legislation, in accordance with the Directives of the European Union. Experiments were performed in male spontaneous hypertensive rats (SHR) weighing 300–325 g (Harlan Laboratories, UK). Rats were kept in a climate-controlled environment on a 12-h light/12-h dark cycle. Food and water were available *ad libitum*. All rats were subjected to an embolic ischemia consisting of a blood embolus placement at the origin of the MCA via a midline neck incision [20]. Arterial blood from a donor rat was withdrawn to form single clots (length: 3 cm; diameter: 0.3 mm) as described [21]. Just before surgery, noninvasively systolic blood pressure was measured with a tail cuff (LE 5002 Storage pressure meter Harvard apparatus, USA) to ensure the hypertension condition of all rats. Continuous laser-Doppler flowmetry (Moor Instruments, Devon, UK) was used to monitor regional cerebral blood flow (rCBF), and only animals that exhibited a reduction >75% during MCAO when compared with rCBF baseline were included in the study. After the surgery, analgesia was administered to the rats (Metamizol, Boehringer Ingelheim, St Cugat del Vallès, Spain).

Animals were anesthetized under spontaneous respiration with 2% isoflurane (Abbot Laboratories, Kent, UK) in oxygen during surgery, and body temperature was maintained at 37°C. All animals were euthanized and transcardially perfused 24 h after the ischemia. Heparine solution followed by saline solution was injected using an infusion pump.

Pilot Study

The aim of our pilot study was to establish a reliable embolic model on hypertensive rats to obtain an adequate rate of HT, our primary endpoint, without increasing the mortality rate. As the time of tPA administration is a critical variable [22], we tested tPA administration at 1.5 and 3 h after MCAO to select the most suitable timing of administration. Animals were euthanized 24 h postischemia, and brains were carefully harvested. Triphenyltetrazolium chloride (TTC) staining was performed to measure the infarct volume, and visual score (0–4) was assessed to evaluate HT (0 = No hemorrhage, 1 = HI-1:Petechial hemorrhage occupying <30% of infarcted area, 2 = HI-2:Petechial hemorrhage occupying >30% of infarcted area, 3 = PH-1:Homogenous blood area occupying <30% of infarcted area, 4 = PH-2:Homogenous blood area occupying >30% of infarcted area).

Experimental Design

Animals subjected to MCAO were randomly allocated to experimental groups (simvastatin+tPA or vehicle+tPA) using a computer-generated randomization list. One milliliter of simvastatin solution (20 mg/kg (diluted in vehicle); Uriach

Laboratories, Barcelona, Spain) or vehicle (distilled H₂O (75%), absolute ethanol (10%) and 0.1 M NaOH (15%)) was subcutaneously injected 15 minutes after occlusion in a blinded manner, and subsequently, rats were allowed to wake up. Animals were reanesthetized 3 h after embolization to receive tPA (Actylise, Boehringer Laboratories) which was slowly infused using an automatic injector in a 9 mg/kg dose. Regional cerebral blood flow (rCBF) was also monitored during the thrombolytic infusion. This timing for tPA administration ensures a high rate of HT without increasing the mortality rate, as established in our pilot study. A total of 50 animals were included in this study. Twelve animals were excluded after applying the following criteria: inappropriate occlusion of the MCA after embolization (*n* = 4); spontaneous reperfusion before tPA administration (*n* = 6) and death before or just after tPA administration (*n* = 2).

Neurological Deficit and Infarct Volume Evaluation

Neurological status was assessed using a 9-point neurological deficit scale in a blinded manner at both 90 min and 24 h after MCAO. This scale consists of four consecutive tests, as previously described [23].

Infarct volume was measured using 2,3,5-triphenyltetrazolium chloride (TTC, Sigma-Aldrich) staining as described [24]. TTC images were captured using a Cano Scan 4200F, and infarct volume was measured using ImageJ software by integration of infarcted areas. Infarct volume data were expressed as a percentage of the ipsilateral hemisphere, and edema was evaluated taking into account the following equation: edema = (infarct volume × contralateral volume)/ipsilateral volume.

Assessment of Hemorrhagic Transformation (HT)

Three different methods were used to evaluate HT severity: (1) classification of HT by visual score, (2) determination of the area of parenchymal hemorrhage (PH), and (3) extravasated hemoglobin (Hb) quantification by Western blot. The antibody used was rabbit anti-Hemoglobin α (H-80, 1:1000, catalog no. sc-21005; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Brain samples used for HT evaluation were obtained after homogenized TTC stained from transcardially perfused rats.

MMP Zymography

Standard gel zymography was used to measure levels of MMP-2 and MMP-9 in both blood and brain samples of ischemic rats. Blood samples were drawn just before tPA administration (3 h postischemia) and before euthanasia (24 h postischemia), collected in EDTA tubs, and immediately centrifuged at 1000 *g* for 10 min to obtain plasma supernatants. For brain samples, frozen coronal brain sections stained with TTC were used to obtain brain homogenates, where describe in detail elsewhere [25]. We previously confirmed that TTC-stain procedure did not modify the MPP-2 and MPP-9 activation status. Both for blood and homogenate brain samples, total protein concentrations were determined with the BCA assay.

Clinical Study

We performed a prospective study of patients with acute ischemic stroke who had been admitted to the emergency room within 3 h after symptom onset. A total of 653 consecutive patients were selected from a large database originally created to study thrombolytic treatment implication on biological and clinical variables. All evaluated patients suffered from a nonlacunar stroke involving the vascular territory of the MCA or the basilar artery. All underwent urgent carotid ultrasound and transcranial Doppler examinations and received tPA (Actylise, Boehringer Laboratories) in a standard 0.9 mg/kg dose (10% bolus, 90% continuous infusion for 1 h). From the 653 patients, 138 were under statins treatment (any kind) at the moment of the stroke, and 515 were not.

Clinical Protocol

A detailed history of vascular risk factors was obtained from each patient with especial attention on the drugs they were taking before stroke onset. To identify possible mechanisms of cerebral infarction, we performed a set of diagnostic tests and classified the cohort according to previously defined etiologic subgroups [26]. Our cohort included 311 cardioembolic strokes, 127 atherothrombotic strokes, 183 patients with an undetermined etiology, 18 dissections, and 14 with uncommon strokes or incomplete study.

Computed Tomography (CT)

All patients underwent CT within the first 3 h of stroke onset. CT was repeated at 24–48 h (or earlier in the case of rapid neurological deterioration) to evaluate the presence of HT. The CT scans were reviewed by a neuroradiologist with extensive experience in acute stroke. The presence and HT type were defined according to previously published criteria [27]. Hemorrhagic infarction (HI) was defined as a petechial infarction without a space-occupying effect, and parenchymal hemorrhage (PH) was defined as hemorrhage with a mass effect. CT-based HT subtypes were defined as: HI-1, for small petechiae along the margins of the infarct; HI-2, for more confluent petechiae within the infarcted area; PH-1, when hematoma involved $\leq 30\%$ of the infarcted area with some slight space-occupying effect; PH-2, when hematoma involved $>30\%$ of the infarcted area with substantial mass effect; or PH-R, when the clot was remote from the infarcted area.

Statistical Analysis

Experimental data were analyzed using GraphPad Prism_v5 software (Graph Pad Prism Software Inc, San Diego, CA, USA) and clinical data using SPSS 15.0 (IBM, New York, NY, USA). Statistical significance for intergroup differences was assessed by Student's *t* test and ANOVA followed by Bonferroni post hoc test for parametric data. For nonparametric data, Mann–Whitney test or Kruskal–Wallis test followed by Dunn's multiple comparison test was assayed. To analyze percentages (mortality rate, hemorrhage incidence, and hemorrhage subtypes in animal study and also patient characteristics), Pearson's chi-square test was used. Bars represent mean $\pm$ SD for parametric data, and box plots represent median (IQR) for nonparametric data. A *P* value < 0.05 was considered statistically significant at a 95% confidence level.

Results

Pilot Study

Surprisingly, we found that SHR without receiving tPA after MCAO showed similar infarct volume than those which received tPA at 1.5 and 3 h postischemia ($P = 0.8$) (Figure 1A). However, we observed significant differences regarding HT rates ($P = 0.016$), although, after Dunn's correction, only when no tPA-treated animals and 3 h tPA-treated animals were compared, the difference reached statistical significance ($P = 0.018$). (Figure 1B). HT's incidence was 57.14% for animals nontreated with tPA, 90% for animal treated at 1.5 h, and 92.30% for those treated at 3 h. Mortality rate was not different among groups.

Simvastatin Efficacy

All animals included in the study showed high arterial systolic pressure and similar weight and occlusion percentage, without significant differences between simvastatin and vehicle-treated groups. Neurological outcome at 90 min (basal) and 24 h post-ischemia also showed no differences between treatment groups (Table S1).

Furthermore, we observed large infarct volumes and different types of intracranial hemorrhages in SHR subjected to embolic stroke, as shown in Figure 2A. No differences were detected on infarct volume percentages between groups: 46.33 (27.58–54.71)%, vehicle+tPA vs. 50(20.09–57.86)%, simvastatin+tPA; $P = 0.91$ (Figure 2B). Correspondingly, rats treated with

Figure 1 Pilot study. (A) Percentage of infarct volume at 24 h of the ischemia expressed as a percentage of the ipsilateral hemisphere, considering no tPA administration (No), tPA administration at 1.5 (1.5 h), and at 3 h (3 h) after the occlusion. (B) Visual hemorrhage score assessed after TTC staining and taking into account tPA administration ($n = 9$ –11 each group).

(A)

(B)

(C)

Figure 2 Simvastatin efficacy. (A)

Representative images of TTC staining where TTC-unstained areas (in white) correspond to infarcted brain tissue from vehicle and tPA-treated rats. Hemorrhages are also evidently seen on the same TTC-stained slices and classified according to ECASS criteria (B) Effect of treatments on infarct volume. Total, cortical, and subcortical size infarcts expressed as percentage of total brain volume in rats subjected to MCAO. Vehicle+tPA ($n = 15$), Simvastatin+tPA ($n = 13$) (C) Graph showing mortality. Bars represent the number of animals that survived or died after 24 h of MCAO.

vehicle+tPA had similar edema volume than those treated with simvastatin+tPA ($114.1 \pm 74.92 \text{ mm}^3$ vs. $121.6 \pm 89.77 \text{ mm}^3$; $P = 0.89$). Furthermore, the mortality rate was high. Twenty-two animals died within 24 h after MCAO, and it was comparable between groups: 10 animals died in the vehicle group (38.46%) and 12 in the simvastatin group (41.37%); $P = 0.77$ (Figure 2C). Regarding CBF reperfusion during the 20 min period of tPA infusion, only few animals showed CBF recovering: 20% of the animals from vehicle+tPA group and 30.85% from simvastatin+tPA group, $P = 0.51$.

Simvastatin Safety

HT was observed in most of the animals independently of treatment allocation at 24 h postischemia (vehicle+tPA = 86.60% vs. simvastatin+tPA = 92.85%, $P = 0.99$). The most frequent hemorrhage identified was PH type (vehicle+tPA = 60% vs. simvastatin+tPA = 71.4%), followed by HI type (vehicle+tPA = 26.6% vs. simvastatin+tPA = 21.4%), but no differences in the incidence of hemorrhage subtype were found among groups ($P = 0.875$) (Figure 3A). Animals treated with simvastatin tended to have smaller PH area as compared with vehicle group ($8.03 \pm 5.69 \text{ mm}^2$ vs.

$20.03 \pm 17.42 \text{ mm}^2$, $P = 0.09$), as shown in Figure 3B. In addition, levels of Hb in ipsilateral hemispheres were not different between groups (18.98 OD ratio [8.62–27.45] vs. 15.46 OD ratio [4.45–23.95], $P = 0.40$) (Figure 3C,D).

Gelatinases Brain and Plasma Content After Simvastatin Treatment

Band intensities of pro-forms of MMP-9 and MMP-2 protein were quantified in gel zymography of brain homogenates to measure gelatinases brain level (Figure 4A). Twenty-four hours after ischemia, pro-MMP-9 levels in brain homogenates were significantly increased in ipsilateral compared with contralateral hemispheres in both vehicle (13.80 [9.20–32.74] A.U. vs. 2.79 [0.01–7.2] A.U., $P = 0.0014$) and simvastatin (20.05 [6.25–30.54] vs. 2.11 [0.01–9.16], $P = 0.0007$) groups. Even so, levels were similar in ipsilateral hemispheres of both groups, $P = 0.52$ (Figure 4B).

On the other hand, we observed a significant increase in pro-MMP-9 OD in the ipsilateral hemisphere of animals that presented hemorrhages (45.00 [34.73–76.26] for HI and 50.21 [6.66–167.30] for PH) in comparison with animals that did not (12.86

Figure 3 Simvastatin safety. (A) Animals' classification according to hemorrhage visual score criteria. Vehicle+tPA ($n = 15$), Simvastatin+tPA ($n = 13$) (B) Quantification of PH areas. Vehicle+tPA ($n = 9$), Simvastatin+tPA ($n = 9$). (C) Representative Western blot for hemoglobin and loading control (β -actin) in ipsilateral (IP) and in contralateral (CL) hemispheres. (D). Vehicle+tPA ($n = 15$), Simvastatin+tPA ($n = 13$).

Figure 4 Matrix Metalloproteinases (MMPs) levels in brain homogenates. **(A)** Representative zymography. **(B)** Levels of MMP-9 proform in ipsilateral (IP) and contralateral (CL) hemispheres. **(C)** Quantification of pro-MMP-9 regarding the hemorrhage type. **(D)** Pro-MMP-9 regarding treatment and hemorrhage type **(E)** Quantification of MMP-2 proform levels. C, loading control. In all graphs of this figure, Vehicle+tPA ($n = 15$), Simvastatin+tPA ($n = 13$).

[6.14–28.71], $P = 0.03$) (Figure 4C). Importantly, we did not found differences between treatment groups (no HT: 9.86 [6.14–15.85] for vehicle and 28.71 [28.71–28.71] for simvastatin; HI's: 57.13 [38.00–76.26] for vehicle and 45.80 [34.73–56.11] for simvastatin; PH's: 45.06 [6.66–140.40] for vehicle and 53.00 [9.99–167.3] for simvastatin (Figure 4D).

Regarding MMP-2 pro-form, similar levels were found in the ipsilateral and contralateral hemispheres within the same group (vehicle: 87.34 [19.96–166.80] vs. 88.06 [24.88–212.60], $P = 0.59$; simvastatin: 132.6 [73.44–229.60] vs. 140.4 [56.30–219.70], $P = 0.77$) and between animals that received simvastatin compared with vehicle treatment ($P = 0.66$) (Figure 4E).

Plasma zymography quantification (Figure 5A) revealed that both pro-MMP-2 and pro-MMP-9 levels were statistically higher at 3 h postischemia compared with 24 hours. (MMP-2 OD ratios: 121.70 [98.54–175.80] vs. 102.00 [68.72–125.00], $P = 0.02$; MMP-9: 135.00 [108.20–249.50] vs. 104.8 [56.76–132.20], $P = 0.006$) (Figure 5B,D). However, no differences were obtained between groups at studied time points (Figure 5C,E).

Statins Treatment and Hemorrhage Incidence in Stroke Patients

Among the recruited patients, only 138 were under statins (21.10%) and 515 were not (78.90%). Importantly, neither differences on HT incidence between both groups ($P = 0.74$) nor among hemorrhage subtype ($P = 0.49$) were found, as shown in Figure 6.

Demographic and clinic characteristics of the stroke population regarding statins treatment were considered (Table S2).

Discussion

As it has been previously published, statins pleiotropic effects could be beneficial even in the acute phase of stroke [28]. It has been demonstrated that statins are able to upregulate endothelial nitric oxide synthase exercising an endothelial protection and to inhibit the upregulation of inducible nitric oxide synthase (mediated by cytokines in astrocytes and macrophages during ischemia and reperfusion), mechanism which results in a cerebral infarct volume decrease [29,30]. Thereon, the main goal of this study was to address the safety concerns related to HT after the combined therapy of statin with tPA. This represents a controversial issue as some studies suggested that statins may reduce HT rates when administered together with tPA [11,12], while others affirmed that the prior statin treatment in stroke patients that received thrombolytic therapy was associated with an increased in HT rate [19] and with a nonfavorable outcome [31,32]. To solve the controversia, a meta-analysis [33] which included 31 randomized controlled trials has been recently published and concluded that statin therapy was not associated with significant increase in ICH.

In our study, we performed a physio-pathological and reliable embolic stroke model in SHR to study the frequency and magnitude of intracranial hemorrhage after cerebral ischemia [34]. The higher vascular resistance in addition to the lower vascular

Figure 5 Matrix Metalloproteinases (MMPs) plasma levels at 3 and 24 h after ischemia. (A) Representative gel zymography. (B) Pro-MMP-2 plasma levels at early and late times of ischemia. (C) Pro-MMP-9 plasma levels at early and late times of ischemia. (D) Pro-MMP-2 plasma expression comparing treatment groups. (E) Pro-MMP-9 plasma expression comparing treatment groups. C, positive control. In all graphs of this figure, Vehicle+tPA ($n = 11$), Simvastatin+tPA ($n = 11$).

Figure 6 Statins safety in stroke patients. Representation of hemorrhage types distribution in tPA-treated patients who were under statin treatment at the moment of the stroke and those who were not. No statins ($n = 515$, 78.9%); statins ($n = 138$, 21.1%).

compliance in hypertensive rats induces larger infarcts, intensifies the vasogenic edema, and aggravates the blood brain barrier (BBB) disruption after ischemia. Consequently, it produces higher frequency of HT in comparison with normotensive animals, in

which HT are less frequent [35]; making this an ideal model to study tPA-induced bleeding complications.

We found that acute simvastatin treatment did not increase the frequency of HT after thrombolytic therapy in SHR. Indeed, the extent of HT evaluated by extravasated hemoglobin showed no differences between vehicle and simvastatin-treated group, and the estimation of PH area indicated even smaller bleedings in animals receiving simvastatin. Regarding MMP data, our results are consistent with previous publications. Stroke produced an acute increase in MMP-9 levels in the ischemic hemisphere at 24 h post-ischemia, which is associated with tPA-related cerebral hemorrhage [36]. Importantly, combined thrombolytic therapy with simvastatin did not further increase MMP-9 levels according to the incidence of HT found, thus supporting the safety of simvastatin when combined with tPA therapy. On the other hand, plasma levels of both MMPs were similarly elevated at 3 h postischemia, preceding tPA administration, in either rats treated with simvastatin or vehicle. A transient elevation in MMP-9 plasma levels during the early reperfusion has been reported in SHR stroke [37], and it has been recently associated with blood-cerebrospinal fluid barrier disruption in SHR [38]. By contrast, we did not observe an ischemic neuroprotective effect exerted by simvastatin treatment in our model. Previous studies performing different stroke models (ligation, intraluminal filament) or using other species and strains (rabbit, mouse, normotensive rats) have shown that simvastatin reduces infarct volume and improves neurological deficit either administered before [39] or after stroke [40]. Therefore, we can

plausible infer that the selected model, with large infarct volumes and hemorrhagic conversions, may abolish the neuroprotective effect of simvastatin in SHR.

In accordance with the results from our pilot study, a recent meta-analysis has also reported that tPA has not significant effect in the infarct volume reduction or in the neurological outcome when administered to ischemic SHR [41]. Their hypothesis is that dilatation of arterioles in response to nitric oxide is mitigated in SHR, which might impair reperfusion after MCAO [42]. To our knowledge, there is only one published study using the embolic stroke model in rats that evaluates the effect of the combined acute treatment of statins and tPA [17]. This work reports that the combined treatment extends the therapeutic window of tPA to 6 h after stroke without increasing HT incidence, and in addition, induces neuroprotection by reducing the infarct volume and improving the neurological outcome. However, the study was performed on normotensive rats, and both their HT rates and infarct volumes were lower than ours. Another study [18] suggests that simvastatin treatment attenuates mechanisms involved in tPA-induced hemorrhage as they demonstrated that the pretreatment with simvastatin significantly decreased tPA-induced hemorrhage incidence when rabbits were subjected to an embolic occlusion. Thus, the protective effect of the statin plus tPA combined treatment assessed in animal stroke models with comorbidities, such as hypertension, has not been demonstrated yet.

Furthermore, the prospective clinical study carried out in our center supports the experimental findings, because statins did not influence HT incidence and severity in stroke patients treated with tPA. Our findings are in the same direction as previous reports that showed that prior statin treatment is an independent predictor of a favorable clinical outcome without an increased frequency of ICH on patients receiving tPA [43]. Contrary, other authors defended that prior statin use is associated with a higher frequency of any intracerebral hemorrhage (ICH) after intraarterial thrombolysis [19]. As these former authors support [19], we also reckon that the different results between the studies can be due to the patient's selection, time to treatment, or treatment modalities (e.g., additional mechanical recanalization). Moreover, it is unknown whether the use of different thrombolytic agents (urokinase and tPA) and different administration routes (systemically or locally) may influence bleeding risk in patients with acute stroke under statin treatment. Additionally, as potential risk factors for ICH (glucose levels, blood pressure) have not been analyzed, the increased risk of ICH found in statin patients group may be the result of those patients being sicker, and therefore, more prone to bleeding compared with patients without statin pretreatment. In this aspect, the patients under statins treatment included in our clinical study showed a significant increase in the leading risk factors related to HT (diabetes mellitus, hypertension) and spite of that, no increase in bleeding complications was identified, reinforcing the safety of the combined treatment. Therefore, only

clinical trials using statins in the acute phase of stroke, such as the ongoing STARS trial (clinicaltrials.gov, identifier: NCT 01073007), will clearly demonstrate the safety and efficacy of the combination of statins plus tPA. If a true effect of statins is demonstrated, treatments might be initiated even before hospital arrival to reach a broader number of stroke patients.

Limitations

Although the animal model chosen might be the most suitable to study HT, we accept this model entails some limitations such as high variability due in part to the different animal response after tPA administration. Moreover, the severity of the experimental model could have not allowed us to see differences regarding HT occurrence between groups or even simvastatin efficacy on infarct volume reduction and neurological improvement.

Despite clinical study supports experimental data, we are aware of the limitations due to the different methodology employed (hypertensive rats receiving a single dose of simvastatin during the acute phase of the stroke vs. hypertension-controlled patients receiving statins chronically before the stroke onset). Regarding the hypertension issue, we wanted to clarify that we use this model in SHR to force high ICH rates that was our main endpoint and not to mimic hypertension related with stroke in humans.

Finally, simvastatin was selected in our experimental study because, as we recently reported in a meta-analysis study [44], it triggers the highest effect associated with stroke neuroprotection among other statins. Nonetheless, other types of statin in combination with tPA may exert similar effects.

In conclusion our study shows that statins administration in the acute phase of stroke (in an experimental/animal study) and as a pretreatment (in a clinical/human study) combined with tPA does not raise HT risk in comparison with administration of tPA alone, supporting the safety of the combined treatment.

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Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

The following supplementary material is available for this article:

Table S1. Physiological parameters, percentage of occlusion and neurological score of animals included in the experimental study.

Table S2. Demographic and clinic characteristics of the stroke population regarding statins treatment included in the clinic study.