

# Alteplase Reduces Downstream Microvascular Thrombosis and Improves the Benefit of Large Artery Recanalization in Stroke

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**Background and Purpose**—Downstream microvascular thrombosis (DMT) is known to be a contributing factor to incomplete reperfusion in acute ischemic stroke. The aim of this study was to determine the timing of DMT with intravital imaging and to test the hypothesis that intravenous alteplase infusion could reduce DMT in a transient middle cerebral artery occlusion (MCAO) rat stroke model.

**Methods**—Rats were subjected to 60-minute transient MCAO. Alteplase (10 mg/kg) was administered 30 minutes after the beginning of MCAO. Real-time intravital fluorescence microscopy through a dura-sparing craniotomy was used to visualize circulating blood cells and fibrinogen. Cerebral microvessel patency was quantitatively evaluated by fluorescein isothiocyanate-dextran perfusion.

**Results**—Immediately after MCAO, platelet and leukocyte accumulation were observed mostly in the venous compartment. Within 30 minutes after MCAO, microthrombi and parietal fibrin deposits were detected in postcapillary microvessels. Alteplase treatment significantly ( $P=0.006$ ) reduced infarct volume and increased the percentage of perfused vessels during MCAO ( $P=0.02$ ) compared with saline. Plasma levels of fibrinogen from alteplase-treated rats showed a rapid and profound hypofibrinogenemia. In vitro platelet aggregation demonstrated that alteplase reduced platelet aggregation ( $P=0.0001$ ) and facilitated platelet disaggregation ( $P=0.001$ ). These effects were reversible in the presence of exogenous fibrinogen.

**Conclusions**—Our data demonstrate that DMT is an early phenomenon initiated before recanalization. We further show that alteplase-dependent maintenance of downstream perfusion during MCAO improves acute ischemic stroke outcome through a fibrinogen-dependent platelet aggregation reduction. Our results indicate that early targeting of DMT represents a therapeutic strategy to improve the benefit of large artery recanalization in acute ischemic stroke. (*Stroke*. 2015;46:3241-3248. DOI: 10.1161/STROKEAHA.115.010721.)

**Key Words:** blood platelets ■ fibrinogen ■ fibrinolysis ■ microcirculation ■ stroke

Intravenous thrombolysis with recombinant tissue-type plasminogen activator (alteplase) has been the therapy of choice for acute ischemic stroke (AIS) within a 4.5-hour time window for 20 years.<sup>1,2</sup> In the past months, the AIS standard of care has changed, with evidence showing that rapid additional endovascular therapy (EVT) was superior to intravenous alteplase alone in the setting of AIS with large vessel occlusions.<sup>3-7</sup> Whether to continue carrying out intravenous

alteplase infusion before EVT has now become a matter of debate.<sup>8</sup> A meta-analysis of EVT showed that the likelihood of a favorable outcome increased with the use of previous intravenous thrombolysis with alteplase.<sup>9</sup> The fact that alteplase infusion exerts beneficial effects even in case of mechanical recanalization suggests that alteplase does not only impact proximal arterial recanalization but also probably prevents or corrects other ischemia/reperfusion-related damage.

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Several experimental studies have examined microvascular changes downstream from focal cerebral ischemia.<sup>10,11</sup> Histological evaluation of the ischemic tissue with light and electron microscopy showed that microvessel lumina were obstructed with platelets, leukocytes, and fibrin-rich aggregates.<sup>12–15</sup> Corroborating these findings, intravital imaging studies have revealed that immediately after arterial recanalization, leukocytes and platelets adhere to endothelial cells in postcapillary microvessels, thus contributing to microvascular obstruction.<sup>16–18</sup> This thrombotic process in microvessels downstream of the initial occlusion site could be responsible for incomplete microcirculatory reperfusion and drive infarct growth despite successful proximal recanalization.<sup>10</sup> Supporting this hypothesis, experimental studies in AIS models have shown that platelet adhesion or aggregation, or leukocyte adhesion inhibition could reduce infarct volume notably by enhancing the patency of downstream cerebral microvessels.<sup>19–22</sup> Furthermore, clinical studies have shown that a state of incomplete reperfusion despite successful recanalization occurs in approximately one quarter of patients with significant impact on clinical outcome.<sup>23,24</sup> Taken together, these data indicate that therapeutic approaches aimed at microvascular obstruction reduction might improve the success rate of recanalization therapies. The exact mechanisms and timing of downstream microvascular thrombosis (DMT) during AIS remain unclear. In particular, no experimental study has explored whether microvascular thrombosis was an early event after proximal occlusion or rather a consequence of ischemia/reperfusion-induced injury. The aim of this study was to determine the timing of DMT in AIS with real-time intravital imaging and to assess the potential efficacy of alteplase to improve microvascular perfusion in a rat model of transient cerebral ischemia.

## Methods

The Animal Ethics Committee of the INSERM-University Paris 7, authorization 2010/13/698-0002, approved animal care and experimental protocols. These experiments were conducted in compliance with the Stroke Treatment Academic Industry Roundtable (STAIR) guidelines.<sup>25</sup>

### Middle Cerebral Artery Occlusion and Reperfusion

Male Sprague–Dawley rats (Janvier, France), weighing 300 to 350 g, were anesthetized by isoflurane mixed with air (4% for induction; 1.5% during surgery), under spontaneous respiration. Focal cerebral ischemia was induced by transient monofilament (4041PK10, Doccol, Redlands, CA) occlusion of the right middle cerebral artery occlusion (tMCAO) for 60 minutes. Animals were randomly assigned to 1 of 2 groups (n=15 per group): control (saline treatment only) and alteplase (10 mg/kg body weight, Actilyse; Boehringer Ingelheim, Ingelheim am Rhein, Germany). Continuous alteplase or saline infusion via the right jugular vein (10% bolus; 90% continuous infusion for 30 minutes; Harvard Apparatus Infusion Pump) started 30 minutes after stroke onset. After monofilament withdrawal, rats were returned to their cages. Body temperature was maintained at 37°C with a heating pad for the duration of surgery. Computer-based randomization was used to allocate drug regimens to each group. Experiments were blinded and the operator was unaware of group allocation during surgery and outcome assessment (Figure I in the online-only Data Supplement).

### DMT Visualization

After a partial dura-sparing craniotomy, rats were subjected to a 60-minute tMCAO (n=10). Intravital fluorescence microscopy

(MacroScope, Leica, France) was used to visualize circulating blood cells (after intravenous injection of rhodamine 6G) and fibrinogen (fluorescein isothiocyanate [FITC] antifibrinogen polyclonal antibody, Dako, France) during MCAO and after recanalization. Microvessel thrombosis was defined as direct visualization of platelet, leukocyte, and fibrinogen aggregates.

### Determination of Infarct Size, Brain Edema, and Hemorrhagic Transformation

Rats were euthanized 24 hours after tMCAO. Brains were quickly removed and cut into 2-mm thick coronal sections using a rat brain slice matrix. The slices were stained with 2% 2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich, St. Quentin Fallavier, France) in PBS to visualize the infarction. Planimetric measurements (Image J software, National Institutes of Health, Bethesda, MD) were performed blinded to the treatment group and were used to calculate infarct volumes and brain edema.<sup>26</sup> The occurrence of hemorrhagic transformation (HT) was macroscopically assessed on whole brains and again after the 2-mm thick coronal brain slices were cut before 2,3,5-triphenyltetrazolium chloride staining.

### Neurological Deficit Evaluation

The neurological deficit was evaluated using a modified Neurological Severity Score, which is a composite of motor, sensory, and balance tests.<sup>26</sup> Neurological function was graded on a scale of 0 to 10 (Figure II in the online-only Data Supplement). Neurological Severity Score was assessed immediately before euthanization at 24 hours after stroke onset.

### Measurements of Microvascular Patency

To examine the patency of cerebral microvessels downstream from MCAO, FITC-dextran ( $2 \times 10^6$  molecular weight, Sigma-Aldrich, France; 1 mL of 50 mg/mL) was administered intravenously to the rats 1 minute before euthanization. The slice at +0.70 mm posterior to bregma was fixed in paraformaldehyde 3.7%. One coronal section from each rat was digitized with a NanoZoomer at  $\times 40$  and analyzed using morphometry software (Histolab 6.1.5, Microvision Instruments). A threshold was applied to each digitized image to ensure that the numbers of FITC pixels reflected the original FITC-dextran-perfused patterns. For each hemisphere, the numbers of FITC pixels divided by the total numbers of pixels were calculated. Data are presented as the ratio between the 2 hemispheres and expressed as a percentage. To test the effect of alteplase on the patency of downstream cerebral microcirculation, microvessels perfused with FITC-dextran were examined 1 hour after MCAO before recanalization in rats treated by alteplase or saline (n=11 per group).

### Measurements of Plasma Fibrinogen Level

Levels of fibrinogen were assayed on plasma samples from immediately before, 1 hour and 24 hours after alteplase or saline infusion. Plasma samples were immediately separated by centrifugation at 2000g for 10 minutes and 2500g for 15 minutes and stored at  $-80^\circ\text{C}$  until further analysis. Fibrinogen levels were measured by the Clauss method.<sup>27</sup>

### In Vitro Platelet Aggregation

Platelet-rich plasma (PRP;  $6 \times 10^8$  platelets/mL) or washed platelets (WP) resuspended in platelet-poor plasma (PPP;  $6 \times 10^8$  platelets/mL) from rats were stimulated with ADP (50  $\mu\text{mol/L}$ ) to induce aggregation. Platelet aggregation was continuously recorded as changes in light transmission (APACT 4004, Elitech France). Four different experiments were performed: platelet aggregation with (1) PRP from rats treated with alteplase or saline; (2) WP resuspended in PPP from rats treated with alteplase or saline; (3) WP resuspended in PPP from alteplase-treated rats with or without addition of exogenous purified rat fibrinogen (Sigma-Aldrich, France); and (4) platelet disaggregation with PRP. Disaggregation of PRP was induced by addition of

alteplase at 3 different doses after the aggregation response reached its maximum.

### Exclusion Criteria

Animals were excluded for analysis if the total lesion volume was <1% ( $n=2$  and  $n=1$  in alteplase and saline group, respectively), if subarachnoid hemorrhage was present ( $n=1$  in each treatment group) or if death because of anesthesia or surgery occurred within 3 hours of stroke induction ( $n=0$ ).

### Animal Sample Size Calculation

The study was designed with 80% power to detect a relative 50% difference in infarct volume between alteplase and saline groups. Statistical testing was performed at the 2-tailed ( $\alpha$ ) level of 0.05 using a  $t$  test. On the basis of preliminary data indicating that the average infarction volume in 60-minute tMCAO was 33%, SD: 15.06%, we used 15 rats for each group.

### Statistical Analysis

All values are presented as mean $\pm$ SD. For statistical analyses, PrismGraph 4.0 software (GraphPad Software, San Diego, CA) was used and differences between the 2 groups were assessed by Mann-Whitney test. Values of  $P<0.05$  were considered statistically significant. The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the article as written.

## Results

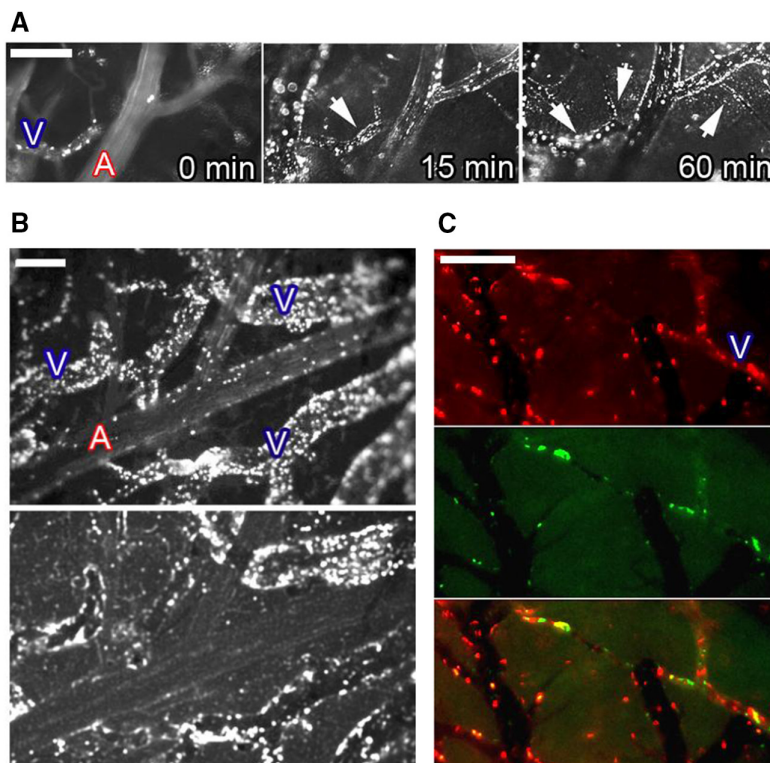
### Transient Monofilament MCAO Induces Early DMT

The timing and frequency of DMT after occlusion of a major cerebral artery were determined using fluorescence intravital microscopy combined with the tMCAO ischemic stroke model. Observation of pial vessels through a partial dura-sparing craniotomy revealed that the accumulation of platelets

and leukocytes started immediately after occluding the MCA (Figure 1A and 1B; Movies I and II in the online-only Data Supplement). This early and pronounced adhesion of leukocytes and platelets occurred almost exclusively in the venous compartment and persisted  $\geq 1$  hour after monofilament withdrawal (Figure 1B; Movie IV in the online-only Data Supplement). Furthermore, in all rats analyzed, during the hour of MCAO, and before MCA recanalization, microthrombosis characterized by intraluminal deposition of fibrin(ogen) developed in postcapillary microvessels at sites of leukocyte and platelet accumulation (Figure 1C). These early aggregates of leukocytes, platelets, and fibrin(ogen) led to a complete cessation of blood flow in at least 1 postcapillary microvessel per field of observation (Figure 1A; Movies II and III in the online-only Data Supplement). After proximal recanalization and despite recovery of blood flow in pial arteries, leukocytes and platelets remained firmly adherent to the postcapillary microvessel wall (Figure 1B; Movie IV in the online-only Data Supplement). These results indicate that DMT is an early event of AIS that principally affects the venous compartment and that can persist despite proximal recanalization.

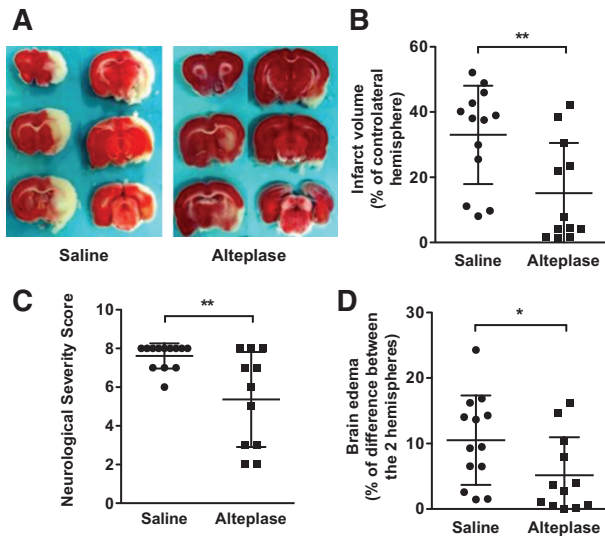
### Alteplase Intravenous Infusion Improves Stroke Outcome After Monofilament Transient MCAO

To determine whether thrombolytic therapy can limit MCAO-induced brain damage, we compared the effect of alteplase and saline on stroke outcome when administered early after monofilament MCAO. Intravenous alteplase injection at 10 mg/kg 30 minutes after initiating tMCAO significantly reduced infarct size evaluated 24 hours later ( $P=0.006$ ; Figure 2A and 2B). Accordingly, the neurological deficit 24 hours after tMCAO was decreased in the alteplase-treated



**Figure 1.** Cerebral microvascular events associated with middle cerebral artery occlusion (MCAO). The cortical microcirculation downstream of the MCA was observed by intravital microscopy before, during, and after MCAO. Platelets and leukocytes were labeled by intravenous injection of rhodamine 6G, and fibrin(ogen) using a fluorescein isothiocyanate-conjugated antifibrinogen polyclonal antibody. **A**, Representative images of rhodamine 6G labeling before (0 minutes), 15 minutes, and 60 minutes after MCAO, as indicated. Immediately after MCAO, platelets and leukocytes started to adhere firmly and to accumulate mostly in the venous compartment, leading to secondary occlusions (arrows). Note the time-dependent increase in occluded microvessels during MCAO. Scale bar, 100  $\mu$ m. **B**, Overview of the microcirculation immediately after MCAO (**top**) and 30 minutes after recanalization (**bottom**). Note that although most of the adherent leukocytes and platelets were flushed away by the arterial flow after recanalization, firmly adherent platelets and leukocytes persisted in veins. Scale bar, 100  $\mu$ m. **C**, Representative image of downstream venous microthrombi. Leukocytes and platelets are in red and fibrin(ogen) in green. The image was taken 30 minutes after MCAO. Scale bar, 50  $\mu$ m. A indicates artery; and V, venule.





**Figure 2.** Infarct volumes, functional outcomes, and brain edema 24 hours after focal cerebral ischemia in rats treated with alteplase or saline. **A**, Representative 2,3,5-triphenyltetrazolium chloride stains of 6 corresponding coronal brain sections of rats treated with saline or alteplase. **B**, Infarct volumes; **C**, modified Neurological Severity Scores; and **D**, brain edema in rats treated with saline (n=13) or alteplase (n=12). \* $P<0.05$ , \*\* $P<0.01$ .

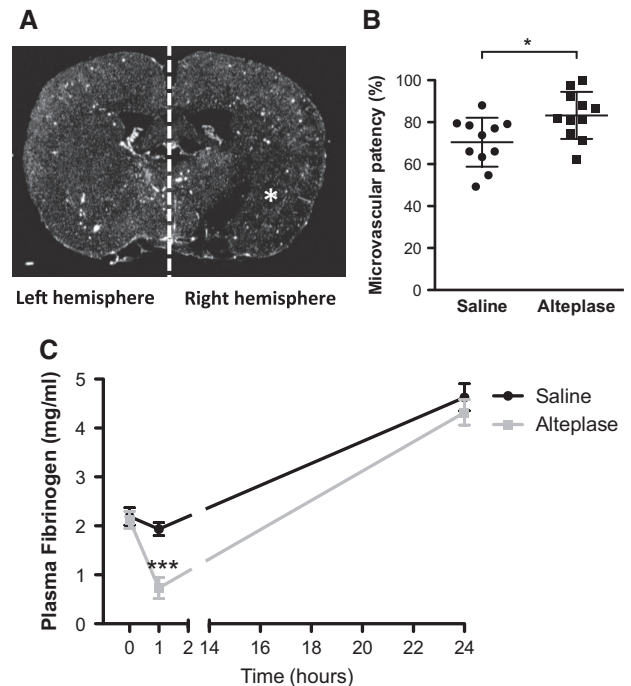
group ( $P=0.004$ ; Figure 2C). Brain edema was also significantly decreased in the alteplase-treated group in comparison with the saline-treated group expressed as percentage difference between the ischemic and nonischemic hemisphere volume (10.5% versus 5.2%, respectively;  $P=0.05$ ; Figure 2D). Importantly, there was no mortality or HT at 24 hours in any group.

The effect of early alteplase treatment on microvascular patency during cerebral ischemia was measured in a subgroup of rats that were euthanized before recanalization at the end of the 60-minute long MCAO period. For these experiments, fluorescent dextran was injected intravenously 1 minute before euthanization to highlight patent vessels. Alteplase treatment led to a significant increase in microvascular patency in the ischemic cerebral hemisphere when compared with saline treatment ( $P=0.02$ ; Figure 3A and 3B). Together, these results showing that early administration of alteplase improves microvascular patency and stroke outcome in a model of tMCAO indicate that alteplase exerts beneficial effects independently of its action on proximal arterial recanalization.

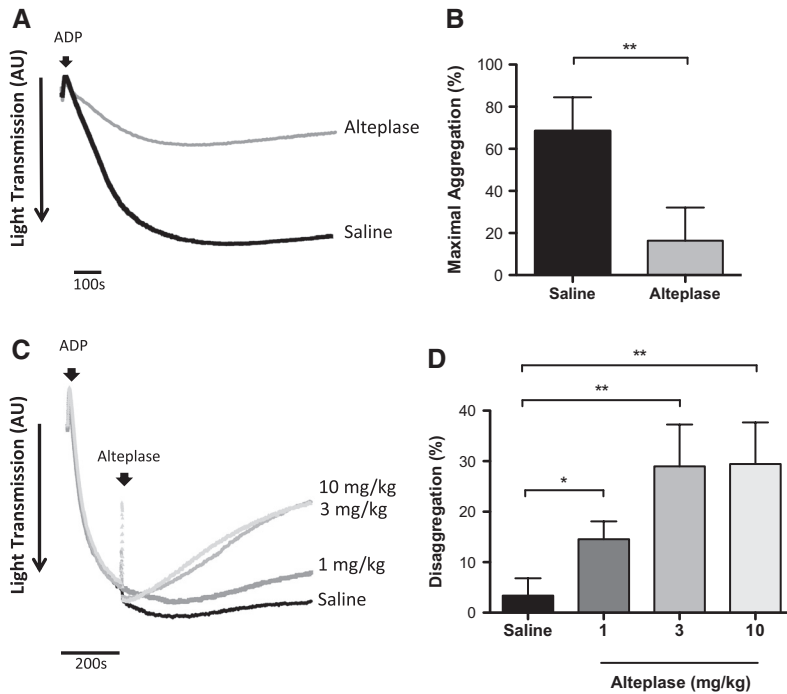
### Alteplase Fibrinolytic Activity Induces a Rapid and Profound Hypofibrinogenemia That Prevents Platelet Aggregation and Promotes Disaggregation of Freshly Formed Platelet Aggregates

The maintenance of microvascular patency by alteplase during MCAO suggested that its early administration might prevent DMT secondary to proximal occlusion. Fibrinogen concentration dramatically decreased at 1 hour after the end of treatment infusion in the alteplase group ( $P<0.001$ ; Figure 3C) but not in the saline group. Furthermore, citrated PRP retrieved from rats treated with alteplase showed a decreased platelet aggregation in response to ADP when compared with PRP

from saline-treated rats (Figure 4A and 4B). In addition, alteplase concentrations corresponding to doses of 1, 3, and 10 mg/kg caused a dose-dependent disaggregation of platelets when added to rat PRP after maximal aggregation response to ADP was achieved (Figure 4C and 4D). To determine whether the effect of alteplase aggregation and disaggregation could be attributed to the alteplase-induced hypofibrinogenemia or whether it further involved a direct effect on platelets through shedding of platelet adhesion receptors, experiments with crossed platelet/plasma systems were performed. WPs and PPP were prepared from rats treated with alteplase or saline and the aggregation response to ADP of both types of WP was tested in either 1 of the 2 PPP. These experiments showed a significant reduction in the aggregation response to ADP of WP, whether from alteplase-treated or control rats, in PPP from alteplase-treated rats when compared with PPP from saline-treated rats (Figure 5A and 5B). Importantly, the addition of exogenous purified rat fibrinogen to PPP from alteplase-treated rats restored the aggregation response to ADP of WP from both alteplase-treated and control rats (Figure 5C and 5D). These results therefore indicate that early administration of alteplase during MCAO helps to maintain downstream microvascular patency by preventing platelet aggregation and promoting platelet disaggregation through induction of fibrinogenolysis.



**Figure 3.** Cerebral microvessels perfused with fluorescein isothiocyanate (FITC)-dextran and changes in plasma fibrinogen level. **A**, Representative image of brain coronal section after FITC-dextran injection 1 hour after middle cerebral artery occlusion in a saline-treated rat. Microvessel patency is decreased in the right hemisphere (\*) compared with the contralateral left hemisphere. **B**, Quantitative data analysis shows percentage of hemisphere microvasculature area perfused with FITC-dextran in rats receiving saline or alteplase. **C**, Changes in plasma fibrinogen level measured by the Clauss method before, 1 hour and 24 hours after saline (n=10) or alteplase (n=10) infusion. \* $P<0.05$ , \*\*\* $P<0.001$ .



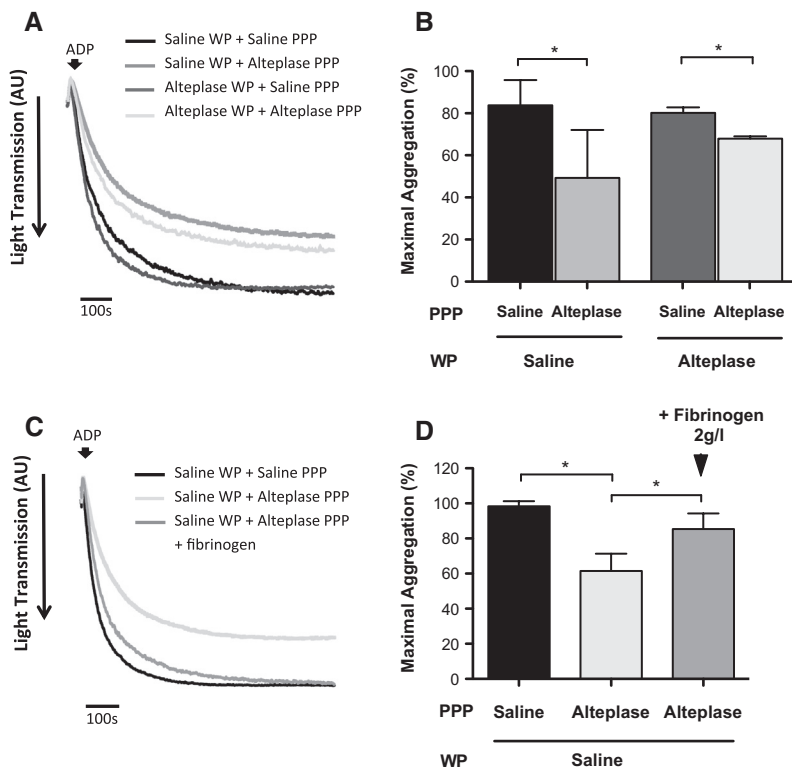
**Figure 4.** In vitro platelet aggregation. Aggregation was induced by addition of ADP (50  $\mu\text{mol/L}$ ), determined by change in light transmission over time and stopped at 600 s. **A**, Representative image of citrated platelet-rich plasma (PRP;  $6 \times 10^8$  platelets/mL) aggregation from rats treated with saline or alteplase. **B**, Maximal aggregation expressed as percentage (%). Data are representative of 3 independent experiments. **C**, Representative image of citrated PRP ( $6 \times 10^8$  platelets/mL) aggregation. Disaggregation of platelets in PRP was induced by addition of alteplase at 1, 3, and 10 mg/kg or saline 200 s after the aggregation response initiation. **D**, Disaggregation was quantified by measuring the ratio between the final and the maximum aggregation for each condition and expressed as percentage. \* $P < 0.05$ , \*\* $P < 0.01$ .

## Discussion

In this study, using a rat tMCAO stroke model combined with in vivo vessel real-time imaging downstream of the occluded MCA, we show that microvascular thrombosis downstream of the proximal occlusion is an early phenomenon that mostly affects the venous compartment, initiated before recanalization. In addition, we show that early intravenous alteplase administration after MCAO is associated with reduced infarct

volume through, at least in part, a fibrinogen-dependent reduction of platelet aggregation, which preserves cerebral microvascular perfusion before recanalization.

Our findings were made possible by the unique features of intravital microscopy when compared with other imaging techniques. We show that DMT starts during arterial proximal occlusion, whereas it has previously been reported to be initiated after reperfusion.<sup>28</sup> In fact, magnetic resonance imaging



**Figure 5.** In vitro platelet aggregation. Aggregation was induced by addition of ADP (50  $\mu\text{mol/L}$ ), determined by change in light transmission over time and stopped at 600 s. **A**, Representative image of platelet-rich plasma (PRP;  $6 \times 10^8$  platelets/mL) aggregation reconstituted with washed platelets (WPs) from saline or alteplase treated rats resuspended in citrated platelet-poor plasma (PPP) from either saline or alteplase-treated rats. **B**, Maximal aggregation expressed as percentage (%). Data are representative of 3 independent experiments. **C**, Representative image of PRP ( $6 \times 10^8$  platelets/mL) aggregation reconstituted with saline-treated WP resuspended in PPP from saline- or alteplase-treated rats and supplemented with exogenous purified fibrinogen at 2 g/L final concentration. **D**, Maximal aggregation expressed as percentage (%). Data are representative of 3 independent experiments. \* $P < 0.05$ .

techniques that evaluate perfusion during proximal occlusion fail to distinguish if microvascular thrombosis affects hypoperfusion or if hypoperfusion is only the consequence of proximal occlusion. Because clinical studies suggested that secondary ischemia and lesion growth would be modest or nonexistent after reperfusion,<sup>29,30</sup> concerns have been raised about whether secondary microthrombosis occurs in patients; and whether it could constitute a therapeutic target for AIS treatment.<sup>28</sup> In this perspective, we demonstrated that DMT is a direct consequence of proximal occlusion and not of reperfusion. Our results suggest a reconsideration of the secondary microthrombosis perception, both in terms of pathophysiology and of how it should be approached as a therapeutic target. In fact, we show that the deleterious impact of DMT on stroke outcome is because of its early consequences on microvessel patency immediately after occlusion. Accordingly, maintenance of microvessel patency during occlusion by early administration of alteplase resulted in infarct size reduction and neurological deficit improvement.

Considering the role of leukocytes, especially neutrophils, in venous thrombosis<sup>31</sup> one could speculate that the risk and intensity of DMT might be enhanced by proinflammatory or procoagulant states (eg, diabetes mellitus, infection, dyslipidemia, etc). For example, diabetes mellitus was recently shown to prime circulating neutrophils for activation and release of procoagulant neutrophil extracellular traps.<sup>32</sup> Thus, early targeting of DMT and the relevance of our findings might be even more important in such comorbid situations.

The therapeutic potential of targeting secondary microthrombosis for AIS treatment was previously suggested by experimental studies where microthrombosis was prevented either by using a constitutive genetic deficiency in thrombus formation in mice or the early administration of antithrombotic drugs after proximal occlusion.<sup>20,21,33–36</sup> Taken together with our findings, these studies indicate that DMT targeting should be achieved as soon as possible following occlusion, even before recanalization, to be effective. In that regard, a recent EVT meta-analysis showed that the likelihood of a favorable outcome increased with the use of previous intravenous thrombolysis with alteplase.<sup>9</sup> Moreover, early alteplase treatment was also shown to predict better outcome despite persistence of large artery occlusion.<sup>37</sup> Our results provide mechanistic insights that could explain these clinical data. Indeed, unlike thrombo-embolic models, the monofilament model of tMCAO mimicks such clinical situations in which the causal occlusion and proximal recanalization are not affected by intravenous thrombolytic treatment. Nevertheless, our findings likely apply to other proximal artery occlusion models of stroke. As we show with intravital microscopy, DMT is not a consequence of reperfusion but of proximal occlusion, an event common to the various large artery ischemic stroke models (transient or permanent MCAO and embolic models). In our experimental model of proximal artery occlusion, despite a profound decrease in downstream blood flow during MCAO, residual perfusion was maintained through collateral anastomosis. This residual blood flow downstream proximal occlusion allows transient tissue survival (penumbra) and regional drug delivery.

Using this model, we demonstrate the pathological importance of DMT and show that it can be overcome by early intravenous alteplase administration that causes a rapid and profound hypofibrinogenemia. The latter events prevent platelet aggregation and help to dissociate freshly formed unstable platelet aggregates. Alteplase-induced hypofibrinogenemia and its consequences on platelet aggregation and disaggregation were previously shown to be due to the incomplete fibrin specificity of alteplase when used at therapeutic plasma concentration.<sup>38–40</sup> This temporary hypofibrinogenemia is also frequently found in patients treated with alteplase. Results from clinical studies have suggested that fibrinogen exerts paradoxical and biphasic effects during the acute phase of ischemic stroke. Elevated initial plasma levels of fibrinogen were shown to be independently associated with a poor stroke outcome.<sup>41</sup> In addition, Ancrod, a purified fraction of venom from Malayan pit viper (*Calloselasma rhodostoma*) that induces a hypofibrinogenemia by splitting the fibrinogen, has been shown to have a favorable benefit-risk profile for patients when administered early within 3 hours of stroke onset.<sup>42</sup> However, other clinical studies have shown that there was no benefit in giving Ancrod beyond 3 hours of stroke onset.<sup>43</sup> Therefore, exactly like for alteplase, Ancrod-induced hypofibrinogenemia seems to be beneficial exclusively when used early after stroke onset. On the other hand, the increased risk of HT associated with hypofibrinogenemia is probably the most important adverse effect of late alteplase treatment.<sup>44,45</sup> Thus, although plasma fibrinogen plays a deleterious role by supporting DMT in the first few hours of AIS, it remains necessary to prevent HT in later stages. Of note, in our model, alteplase was not associated with HT when administered early after occlusion.

In conclusion, our data demonstrate that DMT occurs immediately after MCAO and contributes to MCAO-induced brain damage. We further show that early intravenous alteplase administration, before recanalization, improves ischemic stroke outcome by preserving cerebral microvascular perfusion downstream to the arterial occlusion. This benefit of alteplase was predominantly mediated by a rapid fibrinolytic action. Thus, our results support the use of alteplase treatment before EVT to improve the benefit of large artery recanalization, through targeting of DMT.

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## Disclosures

None.

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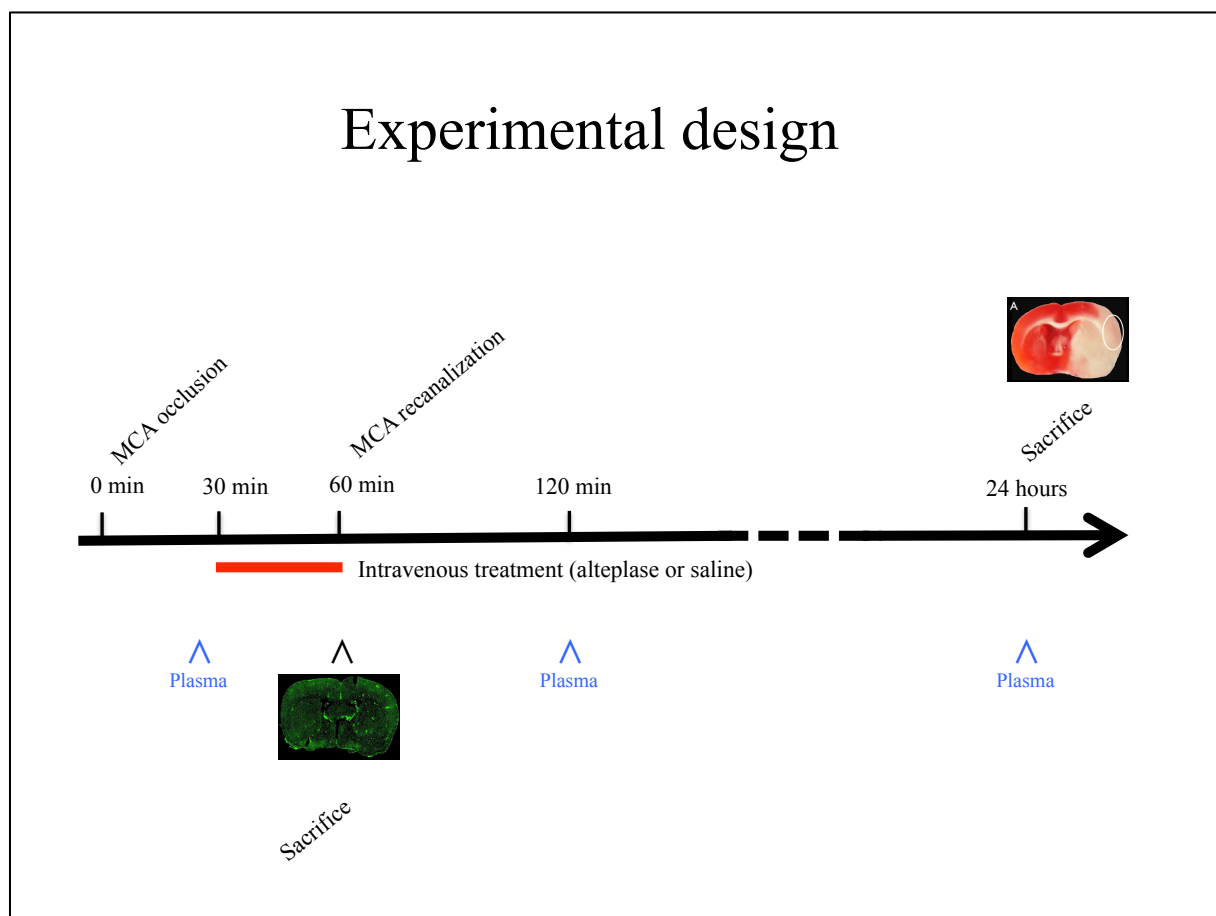
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**Supplemental figure I.** Schematic representation of the experimental design of transient MCAO in rats treated with alteplase or saline.

## SUPPLEMENTAL MATERIAL

<b>Modified Neurological Severity Score.</b>	
<b>Motor tests (<i>raising rat by the tail</i>):</b>	<b>3</b>
1. Flexion of forelimb	
1. Flexion of hindlimb	
1. Head moved > 10° to vertical axis within 30s	
<b>Sensory test (vibrissae stimulation)</b>	<b>1</b>
<b>Beam balance tests</b>	<b>6</b>
0. Balances with steady posture	
1. Grasps side	
2. Hugs and one limb falls down	
3. Two limbs fall down or spins (>60s)	
4. Falls of >40s	
5. Falls of >20s	
6. Falls of <20s	
<b>Maximum points</b>	<b>10</b>

**Supplemental figure II.** Modified Neurological Severity Score.

### **Legends for the Video files:**

**Supplemental Video I.** Pial cerebral microcirculation before MCAO. Leucocytes and platelets were labeled with rhodamine 6G. The video was taken immediately before MCAO. Scale bar = 100 µm.

**Supplemental Videos II and III.** Pial cerebral microcirculation during MCAO. The supplemental Video II was taken 15 minutes after MCAO and supplemental Video III immediately before recanalization (60 minutes after MCAO). Leucocytes and platelets were labeled with rhodamine 6G. MCAO caused an immediate decrease in arterial and venous blood flow. Platelet and leukocyte accumulation were observed mostly in the venous compartment. They formed occlusive microthrombi (arrows) that developed in post-capillary microvessels. Note the time-dependent increase in occluded microvessels during MCAO. Scale bar = 100 µm.

**Supplemental Video IV.** Pial cerebral microcirculation after MCA recanalization. Leucocytes and platelets were labeled with rhodamine 6G. Leukocytes and platelets remained firmly adherent to the post-capillary vessel wall. The video was taken 30 minutes after MCA recanalization. Scale bar = 200 µm.

## **Alteplase Reduces Downstream Microvascular Thrombosis and Improves the Benefit of Large Artery Recanalization in Stroke**

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