Preclinical Evidence Toward the Use of Ketamine for Recombinant Tissue-Type Plasminogen Activator-Mediated Thrombolysis Under Anesthesia or Sedation

Clement Gakuba, MSc*; Maxime Gauberti, MSc*; Mikael Mazighi, MD, PhD; Gilles Defer, MD; Jean-Luc Hanouz, MD, PhD; Denis Vivien, PhD

Background and Purpose—Endovascular treatment of ischemic stroke usually involves recombinant tissue-type plasminogen activator (rtPA)-mediated thrombolysis in anesthetized patients. Paradoxically, differential influences of anesthetic agents on thrombolysis outcome remain unknown.

Methods—In situ thrombotic stroke was induced in mice by local injection of thrombin. Four hours after the ischemic onset, mice underwent rtPA-mediated thrombolysis either awake or subjected to different anesthetic regimens (propofol, isoflurane/N₂O, ketamine). Infarct volume and arterial recanalization were assessed by MRI at 24 hours.

Results—Whatever the anesthetic regimen, infarct volumes measured at 24 hours were not affected. However, in contrast with other anesthetic agents tested, ketamine dramatically reduced infarct volume when combined with rtPA.

Conclusions—Altogether these data suggest that ketamine significantly improves the benefit of rtPA-induced thrombolysis after stroke. 

Key Words: anesthesiology ▪ endovascular treatment ▪ thrombolysis

A recent controversy has emerged in the field of endovascular treatment for stroke.¹ Tremendous efforts have been made in the past decade to design the optimal protocol in interventional radiology to recanalize occluded arteries. Paradoxically, there is no prospective study conducted so far to evaluate the impact of periprocedural anesthetic regimens on stroke outcome. Using an in situ thrombotic stroke model in mice,² we have evaluated the impact of different anesthetic protocols combined with recombinant tissue-type plasminogen activator (rtPA)-mediated thrombolysis on subsequent ischemic lesion sizes (using propofol [Presenius, Sèvres, France], isoflurane [Baxter, Maurepas, France]/N₂O, or ketamine [Virbac, Carros, France]).

Materials and Methods

Animals
Male Swiss mice (30–33 g; CURB, Caen, France) were housed with a 12-hour light/12-hour dark cycle with free access to food and water. Experiments complied with the European Directives and the French Legislation on Animal Experimentation. During 3 weeks, once per day, all animals involved in this study were accustomed to stay awake in 50-mL opaque BD falcon tubes.

Surgical Procedures
We performed in situ thrombotic occlusion in anesthetized mice (2% isoflurane in a mixture of O₂/N₂O 33%/67% with a rectal temperature maintained at 37°C) as previously described by Orset et al.² The operator performing surgery was unaware of the treatment group; 60 minutes after ischemia induction, mice were allowed to recover, and 220 minutes after thrombin injection, mice (excepting the isoflurane/N₂O group) were placed in an opaque tube. Then, 50 µL of 1% lidocaine was injected in the tail to induce local anesthesia, allowing placement of a vein catheter. Thereafter, mice received intravenous rtPA (10 mg/kg, 10% bolus/90% infusion over 40 minutes) or saline. Concomitantly, mice received an intravenous infusion of saline (20 µL in bolus, 180 µL in infusion), propofol (6.6 mg/kg in bolus, 90 mg/kg per hour), or ketamine (3.5 mg/kg in bolus, 47.2 mg/kg per hour). In the isoflurane/N₂O group, mice were reanesthetized (as described) for rtPA or saline intravenous infusion. Propofol and ketamine doses were chosen to achieve conscious sedation (relative immobility with movements induced by stimuli). At the end of the procedure, rectal temperature was measured.

MRI
Experiments were performed 24 hours after ischemia on a Pharmascan 7T (Bruker, Germany) in anesthetized mice (isoflurane/N₂O). T2-weighted images were acquired using a multi-slice multi-echo (MSME) sequence: echo time/repetition time of 51 ms/2500 ms. Two-dimensional time-of-flight angiographies were acquired with
echo time/repetition time of 12 ms/7 ms. Ischemic volumes were quantified on T2-weighted images using ImageJ software (n=6–8 per group).

**Clot Lysis Assay**

Euglobulin fraction of pooled human plasma was resuspended in HEPES buffer; 15 mmol/L calcium chloride and 10 IU of rtPA and/or 80 μg/mL ketamine (when appropriate) were added. Absorbance (405 nm) was monitored during 14 hours at 37°C. Time to achieve 50% clot lysis was measured. Tests were performed in triplicate.

**Statistical Analyses**

Statistical analyses were performed using Kruskal-Wallis followed by Mann-Whitney U test. Statistical significance was concluded for \( P < 0.05 \). Data are mean±SEM.

**Results**

There were no significant differences in cerebral ischemic volumes of thrombolysed animals either awake or anesthetized by propofol or isoflurane/N\(_2\)O (Figure 1). However, administration of ketamine during thrombolysis significantly decreased lesion sizes when compared to awake (−57%), propofol (−56%), and isoflurane/N\(_2\)O (−50%) regimens (with or without thrombolysis, \( P < 0.05 \)) or compared to ketamine alone (−45%; \( P < 0.05 \)). Propofol, isoflurane/N\(_2\)O, or ketamine out of any thrombolysis failed to influence ischemic volumes (Figure 2). Furthermore, reperfusion status measured 24 hours after stroke onset by MR angiography was not influenced by anesthetic regimens (not shown). Moreover, ketamine did not affect rtPA fibrinolytic activity (Figure 3). At the end of thrombolytic procedures, no difference between awake and ketamine-sedated mice temperatures (36°C±0.2 SEM) was observed, and only a slight, but significant (\( P < 0.05 \)) decrease for propofol-sedated mice (35°C±0.2 SEM) was observed. Ketamine sedation did not induce hypothermia in continuous temperature-monitored mice in an independent control group (not shown).

**Discussion**

We report for the first time to our knowledge the lack of difference in ischemic volumes at 24 hours between rtPA-induced thrombolysis performed in awake animals or under propofol or isoflurane/N\(_2\)O anesthesia. The main result is the demonstration that infusion of ketamine during rtPA-induced thrombolysis led to a dramatic reduction of ischemic lesions when compared to nonanesthetized animals or to other anesthetic regimens performed.

There are 2 main classes of general anesthetics used in clinical settings. The first group potentiates the GABAergic neurotransmission (such as isoflurane and propofol), whereas the second inhibits glutamatergic neurotransmission (such as ketamine). In the present study, we evidence that neither GABAergic nor antiglutamatergic anesthetics reveal neuroprotective potential when applied alone far from cerebral ischemia. Only association of ketamine with rtPA led to reduced ischemic lesions. One possible explanation for this differential property of ketamine may be related to the proexcitotoxic effects of rtPA.³ We have previously reported that rtPA increased infarct volumes when injected 4 hours...
after ischemic onset. Without concomitant treatment, proexcitotoxic effects of rtPA overcome benefits of late rtPA-induced reperfusion. Ketamine, reported as an NMDA receptor antagonist, could prevent rtPA-promoted NMDA receptor-mediated excitotoxicity, thus unmasking benefits of thrombolysis. In the present experimental model, benefits of early rtPA-mediated thrombolysis, 20 minutes after the onset, has been demonstrated. However, as in clinical practice, delayed administration of rtPA fails to afford neuroprotection. Because revascularization during endovascular approaches often occurs in the limits of the therapeutic window, the choice of the anesthetic strategy may positively impact time-to-treatment opportunities. It should be pointed out that ketamine is a common and widely used anesthetic agent without any known side effects in neuroanesthesia.

We must, however, expose some limits of the present data. First, volume lesion in rodents is an intermediary end point, and thus our findings need to be confirmed by functional testing in higher species. Second, because of interspecies differences in fibrinolytic systems, rtPA dose was 10-times higher on a per-weight basis than the dose used in clinical practice. Whether this would influence rtPA—ketamine interaction remains to be investigated.

Conclusions
In conclusion, our results suggest that use of ketamine must be considered when anesthesia and/or sedation are required to perform thrombolysis, because it reveals synergistic effects when combined with rtPA-induced thrombolysis. A prospective clinical trial is nevertheless needed to validate this hypothesis.

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Disclosure
None.

References
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