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ORIGINAL RESEARCH

Comparative Effects of Glenzocimab and Eptifibatide on Bleeding Severity in 2 Mouse Models of Intracranial Hemorrhage

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BACKGROUND: Antiplatelet drugs represent potential candidates for protecting the penumbral microcirculation during cerebral ischemia and improving the benefits of arterial recanalization in ischemic stroke. Yet while the efficacy of such adjuvant strategies has been shown to be highly time dependent, antiplatelet therapy at the acute phase of ischemic stroke cannot be envisioned until the diagnosis of stroke and its ischemic nature have been confirmed because of the presumed risk of worsening bleeding in case of intracranial hemorrhage (ICH). Here, we investigated this risk for 2 antiplatelet drugs currently being tested in clinical trials for ischemic stroke, glenzocimab and eptifibatide, in 2 mouse models of ICH.

METHODS AND RESULTS: The severity of ICH was assessed in mice humanized for glycoprotein VI treated or not with glenzocimab or eptifibatide at effective dose, in a model of primary ICH caused by unilateral striatal injection of collagenase type VII, and in a model of hyperglycemia-induced hemorrhagic transformation of cerebral ischemia–reperfusion injury. Glenzocimab had no impact on bleeding severity in either model of ICH. Conversely, eptifibatide caused a significant increase in intracranial bleeding in both models, and a drastic increase in death after hyperglycemia-induced hemorrhagic transformation of cerebral ischemia–reperfusion injury.

CONCLUSIONS: Unlike eptifibatide, glenzocimab is safe in the setting of ICH. These results suggest that glenzocimab could be administered upon suspicion of ischemic stroke, before assessment of its ischemic nature, thus opening the way to hastening of treatment initiation.

Key Words: antiplatelet drugs ■ intracranial hemorrhage ■ ischemic stroke ■ platelets ■ safety

Besides being crucial components of ischemic stroke (IS) thrombi, platelets promote infarct growth through their participation in the ischemia-induced microvascular thromboinflammatory response, which leads to secondary occlusions in collaterals and to progressive penumbral tissue loss.[1,2] This has made antiplatelet drugs potential candidates for adjuvant therapy aimed at improving the benefits of recanalization by dampening thromboinflammation and protecting the penumbra. Yet, because of the crucial involvement of platelets in hemostasis and limitation of intracranial hemorrhage (ICH),[3] administration of antiplatelet drugs at the hyperacute phase of IS cannot be envisioned until the diagnosis of IS and the absence

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CLINICAL PERSPECTIVES

What Is New?

- Administration of antiplatelet agents for acute ischemic stroke is often delayed by the requirement of imaging to rule out intracranial hemorrhage.
- We assess the hemostatic safety of 2 glycoprotein IV inhibitors in 2 mouse models of intracerebral hemorrhage.
- We show that the hemostatic safety of glenzocimab is maintained in the context of intracranial bleeding, while eptifibatide increased intracranial bleeding in mouse models.

What Are the Clinical Implications?

- These results provide animal model data to support design of clinical trials of accelerated intervention in suspected acute ischemic stroke with glenzocimab.

Nonstandard Abbreviations and Acronyms

ACTIMIS	Acute Ischemic Stroke Interventional Study
ACTISAVE	Acute Ischemic Stroke Study Evaluating Glenzocimab Used as Add-on Therapy Versus Placebo
ANGEL-ACT	Endovascular Treatment Key Technique and Emergency Work Flow Improvement of Acute Ischemic Stroke
EPOCH	Eptifibatide in Endovascular Treatment of Acute Ischemic Stroke
GPVI	glycoprotein VI
GREEN	Glenzocimab for Reperfusion in the Setting of Endovascular Therapy for Brain Infarction
HT	hemorrhagic transformation
ICH	intracranial hemorrhage
IS	ischemic stroke
MCA	middle cerebral artery
tMCAO	transient middle cerebral artery occlusion

of brain hemorrhage have been confirmed by imaging. This safety-related constraint delays the possibility of antiplatelet treatment initiation, therefore reducing its potential benefits. Indeed, recent studies have shown that microvascular thromboinflammation is an immediate response to cerebral ischemia,[1] whose deleterious consequences are progressive and can be slowed down by early pharmacological intervention, before arterial recanalization.[2,4,5] Moreover, there is evidence that the efficacy and safety of antiplatelet strategies in IS are highly time dependent: Whereas immunodepletion of platelets is protective in the mouse model of monofilament-induced cerebral ischemia–reperfusion when induced before middle cerebral artery (MCA) occlusion,[6] it causes massive hemorrhagic transformation (HT) when induced after arterial MCA recanalization.[7,8] While these observations illustrate the risk of hemorrhagic complications of antiplatelet therapy in IS, this risk may not be inherent to all antiplatelet drugs.

Glenzocimab is a novel antiplatelet drug consisting of a humanized antibody fragment targeting platelet glycoprotein VI (GPVI),[9] the main platelet receptor for collagen, but also a ligand for fibronectin, vitronectin, laminin, and an important mediator of fibrin-induced procoagulant activity of platelets.[10] Interestingly, glenzocimab has been demonstrated to block GPVI function and to be antithrombotic in a variety of models.[9,11–13] The interest of targeting GPVI in IS has been suggested by previous studies in mouse models of cerebral ischemia–reperfusion in which it was shown that pharmacological targeting of GPVI reduces infarct progression before and after arterial recanalization.[5,14] Furthermore, from a safety standpoint, to date, neither GPVI deficiency nor pharmacological inhibition of GPVI, including with glenzocimab,[7] causes HT of IS in nonhemorrhagic mouse models of cerebral of ischemia–reperfusion.[5,14,15] Consequently, glenzocimab has entered several clinical trials for IS (ie, ACTIMIS [Acute Ischemic Stroke Interventional Study], NCT03803007; ACTISAVE [Acute Ischemic Stroke Study Evaluating Glenzocimab Used as Add-on Therapy Versus Placebo], NCT05070260; and GREEN [Glenzocimab for Reperfusion in the Setting of Endovascular Therapy for Brain Infarction], NCT05559398). Remarkably, the phase 1b/2a ACTIMIS trial was recently completed and indicated that glenzocimab combined with intravenous thrombolysis alteplase, with or without mechanical thrombectomy, was well tolerated.[16]

Eptifibatide is an antagonist of the main platelet receptor for fibrin(ogen), glycoprotein IIb/IIIa. Despite the central role of glycoprotein IIb/IIIa in platelet aggregation and hemostasis, several clinical studies have suggested that, like glenzocimab, eptifibatide might be safe when administered in combination with intravenous thrombolysis[17,18] or endovascular therapy (ie, EPOCH [Eptifibatide in Endovascular Treatment of Acute Ischemic Stroke], NCT03844594; ANGEL-ACT [Endovascular Treatment Key Technique and Emergency Work Flow Improvement of Acute Ischemic Stroke], NCT03370939)[19–23] for the treatment of acute IS caused by large-vessel occlusion.

The reassuring safety data regarding the use of glenzocimab and eptifibatide in patients with IS provide clinical evidence that targeting the thromboinflammatory

cascade with antiplatelet drugs is not intrinsically associated with an increased risk of HT when used in combination with standard-of-care recanalization therapy in carefully selected patients showing no bleeding on imaging. Interestingly, the absence of impact of glenzocimab in bleeding time assays in mice and humans[9,13] further suggests that it might be safe even in case of ICH, which may render exclusion of ICH before treatment initiation dispensable and thus hasten therapeutic intervention. Nonetheless, whether the safety profiles of glenzocimab and eptifibatide are maintained in the context of ICH remains unknown. Here, we investigated the safety of glenzocimab and eptifibatide in 2 different mouse models of ICH.

METHODS

Data Availability Statement

Data will be made available upon reasonable request to the corresponding author.

Animals

All animal procedures described in this study were performed using male mice 8 to 12 weeks old (25 ± 5g). Mice humanized for GPVI,[13] as well as GPVI$^{-/-}$ mice and their wild-type GPVI$^{+/+}$ littermates,[24] were bred and housed in our animal facility. Experiments were conducted according to the French veterinary guidelines and those formulated by the European Community for experimental animal use (directive 86/609 EEC), and were approved by our local committee on the ethics of animal experiments (APAFIS 12122).

ICH Model by Injection of Type VII Collagenase

Primary ICH was induced by injection of type VII collagenase as described previoulsy.[25,26] Briefly, mice were shaved at the skull, injected with buprenorphine (0.1 mg/kg) before surgery, and anesthetized using isoflurane gas inhalation (4% for induction, 1.5% for maintenance). Mice were fixed in a prone position on a brain stereotaxic frame under a dissecting magnifier, and a craniotomy was performed with a skull drill, while brain moisturization was maintained with a compress soaked in artificial cerebrospinal fluid (87 mM NaCl, 25 mM $NaHCO_3$, 75 mM sucrose, 10 mM glucose, 2.5 mM KCl, 1mM NaH2PO$_4$, 7 mM $MgCl_2$, pH 7.4). Unilateral striatal injection of type VII collagenase (0.05 U in 0.5 µL) was performed using a thin glass pipette (coordinates from bregma: *x*: 2 mm, *y*: 0 mm, *z*: 3.5 mm). Glenzocimab (gift from Acticor Biotech) and eptifibatide (Sigma-Aldrich; SML1042-50) were administered randomly 15 minutes before surgery. To benefit from both the immediate action of the intravenous route and the prolonged action of the intraperitoneal route,[27] for each drug, half of the dose was administered intravenously, and the other half intraperitoneally The platelet-depleting antibody R300 to mouse glycoprotein Ibα (4 µg/g mouse; Emfret Analytics) was injected through the retro-orbital plexus 15 minutes before surgery. Mice were euthanized by intracardiac perfusion of cold artificial cerebrospinal fluid under ketamine/xylazine anesthesia, 2.5 hours after collagenase injection. Brains were collected, sliced with a razor blade, and stored at −80°C for preparation of brain homogenates for assessment of hemoglobin content.

Hyperglycemia-Induced Hemorrhagic Transformation of Cerebral Ischemia–Reperfusion

Mice were shaved at the neck, and buprenorphine (0.1 mg/kg) was injected subcutaneously before surgery. Under anesthesia by isoflurane (5% for induction, 2% for maintenance) delivered in oxygen, mice were subjected to transient middle cerebral artery occlusion (tMCAO; 60 minutes) with a monofilament inserted through the common carotid artery, as described previously.[7,28] A Doppler probe (Moor Instruments Ltd, Millwey, UK) placed on the temporal bone was used to monitor cerebral blood flow in the MCA territory preoperatively and after insertion of the monofilament. All mice included in this study had a drop in cerebral blood flow in the MCA territory of at least 70% following monofilament insertion in the MCA. Glucose (30% in saline) was administered at 2.2 g/kg IP, 15 minutes before MCA occlusion.[29] Glycemia was verified by tail sampling using a blood glucometer (CALLA Light; Wellion).

Glenzocimab (64 mg/kg) and eptifibatide (50 mg/kg) were administered randomly at the time of recanalization by combined intravenous and intraperitoneal routes. Three hours after recanalization, glenzocimab or eptifibatide were reinjected to prolong the drug action. Mice were euthanized by intracardiac perfusion of cold artificial cerebrospinal fluid under ketamine/xylazine anesthesia, 6 hours after recanalization. Brains were collected, sliced with a razor blade, and a mouse brain slice matrix, and hemispheres were separated and stored at −80°C for preparation of brain homogenates.

Whole Blood Platelet Aggregometry

Whole blood impedance aggregometry was performed using a multiplate analyzer (Roche, Rotkreuz, Switzerland) following the manufacturer's instruction. Mouse blood was collected in 0.1 M citrate (citrate/blood: 1/9, v/v), allowed to stand at room temperature for 30 minutes, before being stimulated with 6.6 µMADP in saline supplemented with 3 mM $CaCl_2$ at

37 °C (citrated blood/calcium saline: 1/1, v/v) to trigger aggregation.

Flow Chamber Experiments

Microfluidic flow chambers (Vena8 Fluoro+, Cellix) were coated overnight at 4°C with 50 μg/mL Horm collagen (Takeda, Linz, Austria) diluted in saline, rinsed with saline, blocked with 10% BSA for 30 minutes, and rinsed again with saline. Mouse whole blood was collected in heparin (heparin/blood: 1/9, v/v), stained with 3,3′-dihexyloxacarbocyanine iodide (Thermo Fisher Scientific, 5 μM), and perfused through the chamber channels at arterial shear rate (1500 s^{-1}) for 3 minutes. Following perfusion, the channels were rinsed with saline perfused for 2 minutes, and 5 representative images were acquired along the channel for quantification of the platelet-positive area using ImageJ (National Institutes of Health, Bethesda, MD).

Quantification of Intracranial Bleeding

Brains were collected, weighed, and homogenized by mechanical disruption using scissors in cold sucrose artificial cerebrospinal fluid (5 Ml/mg tissue) supplemented with 0.1% NP40 and protease inhibitor cocktail (R&D Systems, Minneapolis, MN), followed by 3 snap freezing–thawing cycles, and sonicated. Homogenates were then centrifuged for 20 minutes at 14000*g* and 4°C, and the supernatants were collected and stored at −80°C until analysis.

Hemoglobin content in brain homogenates was quantified using a formic acid–based assay for heme[30,31] for the model of type 7 collagenase–induced ICH, and by a more sensitive mouse hemoglobin ELISA (Mouse Hemoglobin; Abcam; ab157715), for the model of hyperglycemia-induced HT of cerebral ischemia–reperfusion.

Statistical Analysis

For both models of ICH, power analyses based on our preliminary data and on results from previous studies[32–35] were performed to determine the necessary sample size, with an α risk set at 5% and a power at 80%. The SD for the model of collagenase VII–induced ICH was estimated at 20% and that of the model of hyperglycemia-induced HT of cerebral ischemia–reperfusion at 35%. Considering the reported impact of eptifibatide and glenzocimab on bleeding time and in models of thrombosis,[7,9,11,13,36–39] calculations were made for detecting increases in bleeding severity of at least 50%. For these parameters, minimal group sizes of 7 were used for the model of hyperglycemia-induced HT of ischemia–reperfusion and group sizes of 3 for that of collagenase VII–induced ICH.

Statistical significance of differences in mean between groups was calculated by the exact 2-sample Fisher-Pitman permutation test using an online calculator (https://kenrice.shinyapps.io/PermutationOneWayTests/). Survival rates were analyzed by comparing Kaplan–Meier curves using the log-rank Mantel–Cox test.

RESULTS

Platelet-Dependent Cerebral Hemostasis in a Model of Primary ICH Is Not Affected by Glenzocimab but Compromised by Eptifibatide

Because the role of platelets in the model of intrastriatal collagenase-induced ICH[25,26] has not been

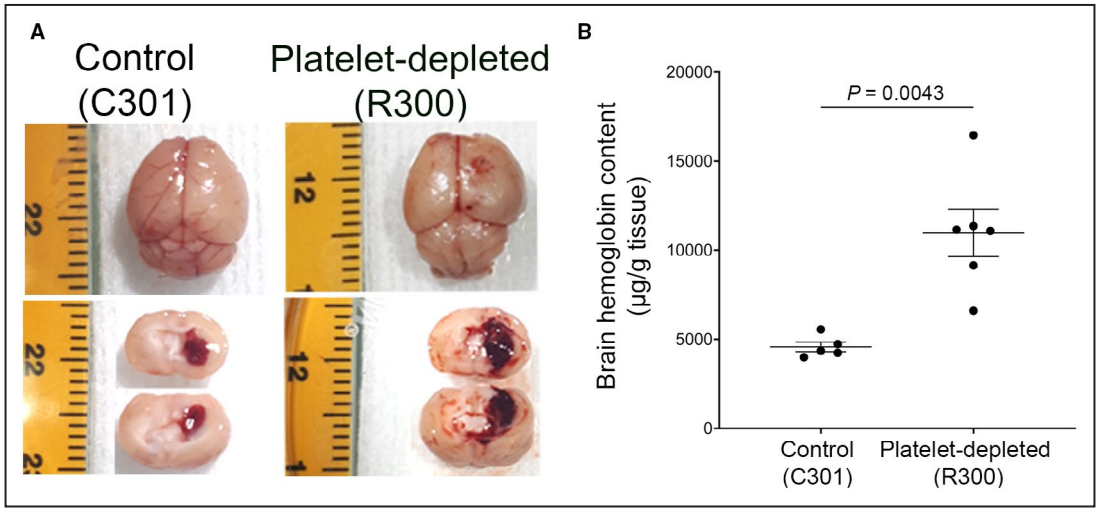

Figure 1. Platelets intervene early to limit collagenase VII–induced intracranial hemorrhage in mice.
A, Representative images of macroscopically visible intracranial bleeding 2.5 h after intrastriatal collagenase VII injection (0.1 IU in 1 μL) in mice treated with a control (C301) or platelet-depleting antibody (R300). **B**, Quantification of intracranial bleeding as estimated by measurement of intracerebral heme content. n=5–6.

investigated, we first determined the impact of platelet depletion in this model of primary ICH. All platelet-depleted mice became lethargic and died within 2.5 hours of collagenase injection, whereas no deaths had occurred in mice with normal platelet count within this time window. The autopsy revealed no extracranial bleeding in either platelet-depleted mice or control mice euthanized 2.5 hours after collagenase injection; however, it confirmed the systematic occurrence of a single, massive, macroscopically visible, intracranial hematoma in both groups of mice (Figure 1). The macroscopic aspect of brains recovered from platelet-depleted mice indicated an increased ICH severity as compared with control mice, which was confirmed by quantification of brain hemoglobin content (Figure 1). Altogether, these data show that platelets intervene early and crucially to limit bleeding and prevent death in this model of collagenase-induced primary ICH.

Notably, no difference in ICH severity was found between 2.5 hours and 24 hours after collagenase VII injection in control mice with normal platelet count, indicating that bleeding had stabilized early (Figure S1 and S2). Therefore, considering the early death of platelet-depleted mice, for ethical reasons, the mice were euthanized no later than 2.5 hours after collagenase injection.

We next assess whether glenzocimab and eptifibatide modified ICH severity. Because glenzocimab specifically targets human GPVI, transgenic mice expressing the human GPVI[13] were used for these experiments. Eptifibatide used at a dose of 10 mg/kg, which was previously shown to fully block platelet aggregation and thrombosis in mice for at least 30 minutes,[36,40] caused a significant worsening of collagenase-induced ICH (Figure 2A and 2B). In contrast, glenzocimab used at a dose of 64 mg/kg, which blocked GPVI-dependent platelet activation for up to 3 hours (Figure 3), did not worsen collagenase-induced ICH (Figure 2A and 2B). The safety of GPVI targeting ain this model of primary ICH was further verified using mice with complete genetic deficiency in GPVI ($GPVI^{-/-}$ mice). $GPVI^{-/-}$ mice showed similar bleeding as their wild-type littermates (Figure 2C and 2D).

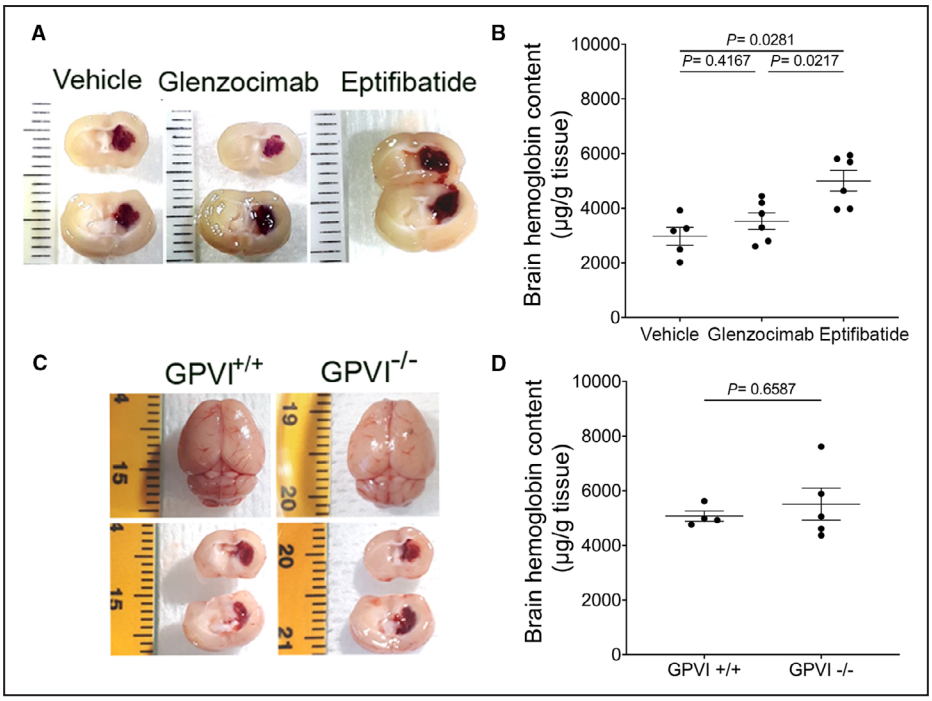

Figure 2. Impact of glenzocimab and eptifibatide in a mouse model of primary intracranial hemorrhage.

A, Representative images intracranial bleeding 2.5 h after intrastriatal collagenase VII injection in transgenic mice expressing human glycoprotein VI and treated with glenzocimab (64 mg/kg) or eptifibatide (10 mg/kg) administered as a bolus (50% intravenously, 50% intraperitoneally) 15 min before surgery. Vehicle corresponds to a mixture of saline with citrate buffer. **B**, Quantification of intracranial bleeding as estimated by measurement of intracerebral heme content 2.5 h after collagenase VII injection. n=5. **C**, Representative images of macroscopically visible intracranial bleeding 2.5 h after intrastriatal collagenase VII injection (0.1 IU in 1 µL) in $GPVI^{+/+}$ and $GPVI^{-/-}$ mice. n=5. **D**, Quantification of intracranial bleeding as estimated by measurement of intracerebral heme content.

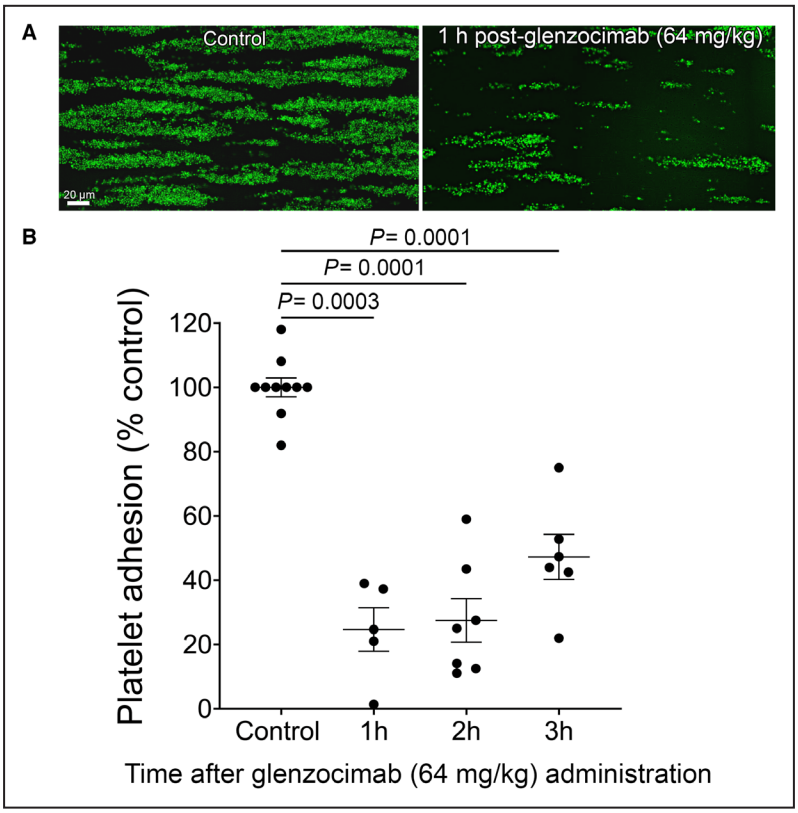

Figure 3. Ex vivo evaluation of the duration of glycoprotein VI inhibition by glenzocimab.
Mice humanized for glycoprotein VI were injected with glenzocimab (64 mg/kg, half of the dose intravenously and the other half intraperitoneally), blood was collected on citrate at different times after injection, labeled with the fluorochrome 3,3'-dihexyloxacarbocyanine iodide, and perfused at a wall shear rate of 1500 s^{-1} for 3 min over a collagen-coated surface. **A**, Representative images of platelet coverage at the end of the perfusion. **B**, Mean surface areas covered by platelets calculated from a minimum of 20 different fields taken with a 63× objective along channels from 4 different runs (5 fields per run, each run from a different mouse). Results are expressed as percentages relative to the mean surface areas covered with platelets in channels perfused with control blood.

Differential Effect of Glenzocimab and Eptifibatide on Cerebral Hemostasis in a Model of Hemorrhagic Transformation of Cerebral Ischemia–Reperfusion

The impact of glenzocimab and eptifibatide on cerebral hemostasis was further investigated using a model of ICH secondary to ischemia–reperfusion. As in patients with stroke,[41–45] hyperglycemia has been shown to increase the rate of HT and to worsen IS outcome, including death, in rats and mice subjected to transient middle cerebral artery occlusion (tMCAO).[35,46–49] In agreement with these data, in preliminary experiments with wild-type mice, we found that combining 60-minute tMCAO with acute hyperglycemia led to 100% HT and 27% death at 24 hours after MCA recanalization. All surviving mice displayed lethargy and had reached humane end points, thus requiring us to euthanize the mice at an earlier time point. While no HT was observed in mice euthanized at the end of the 1-hour-long MCA occlusion period, HT under the form of petechiae was systematically found in mice euthanized 6 hours after MCA recanalization (Figure S2), which was therefore set as the time point for euthanasia in the experiments described hereafter. Notably, because these data further indicated that the onset of HT in hyperglycemic mice subjected to tMCAO occurred within 6 hours of MCA recanalization, the eptifibatide dose was increased from 10 mg/kg to 50 mg/kg to cover a longer time span. The latter eptifibatide dosage completely blocked platelet aggregation for at least 1 hour, but the inhibition faded at 3 hours after treatment, as assessed by ex vivo whole blood aggregometry (Figure 4). As a result, in addition to administration of eptifibatide at recanalization, a second dose of eptifibatide was administered 3 hours after MCA

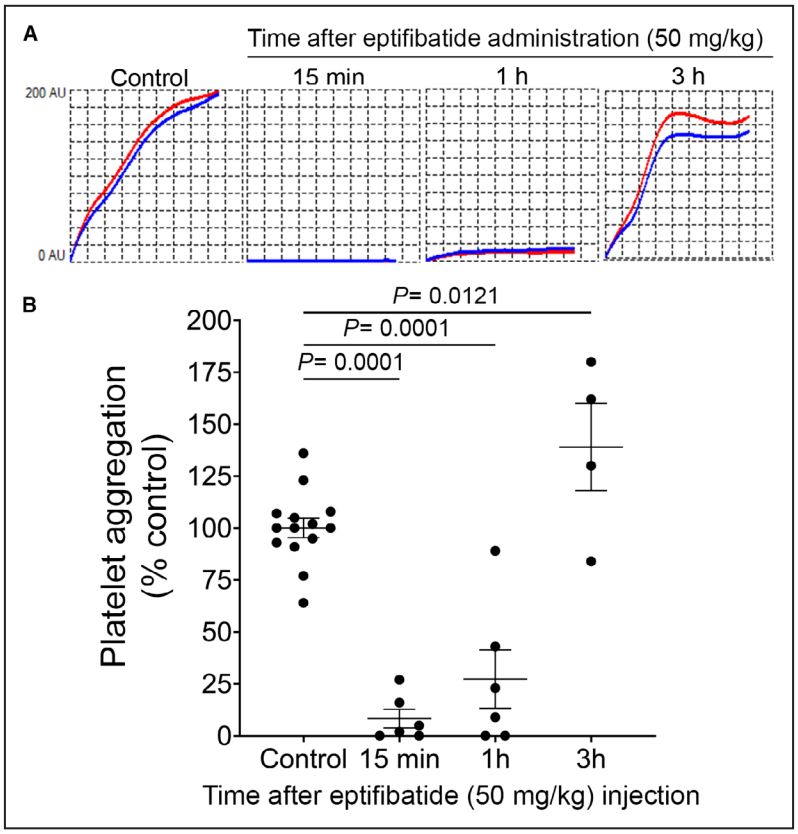

Figure 4. Ex vivo evaluation of the duration of αIIbβ3 integrin inhibition by eptifibatide.
Mice were injected with eptifibatide (50 mg/kg, half of the dose intravenously and the other half intraperitoneally), blood was collected on citrate at different times after injection, and whole blood impedance aggregometry was performed using a multiplate analyzer. **A**, Representative whole blood aggregometry curves obtained in response to 6 μM ADP according to blood collection time after eptifibatide treatment. **B**, Mean platelet aggregation expressed as percentages relative to the mean area under the aggregation curve obtained with blood from control mice injected with saline. n=4–13 mice per group.

recanalization. The same administration protocol was used for glenzocimab, as it also showed a decrease in its inhibitory activity at 3 hours after MCA recanalization (Figure 3).

In all groups of mice, glucose injection led to an acute and temporary rise in nonfasting blood glucose levels from ≈200 mg/dL at baseline to 400 mg/dL at the time of MCA occlusion, before returning to baseline levels at recanalization (Figure 5A). As in wild-type mice (Figure S2), all mice humanized for GPVI subjected to tMCAO under hyperglycemia had developed HT at 6 hours after recanalization (Figure 5B). Glenzocimab did not modify HT severity or death (Figure 5B through 5D). Conversely, eptifibatide caused an increase in death, with 4 of 9 mice that had died within 6 hours of MCA recanalization (Figure 5D), in association with a significant increase in HT severity (Figure 5B through 5D). As in the model of primary ICH, the safety of GPVI targeting in this model of HT was confirmed in GPVI$^{-/-}$ mice, which showed similar bleeding as their wild-type littermates (Figure 5E and 5F).

DISCUSSION

Over the past decade, the development of mechanical thrombectomy for arterial recanalization has been a major breakthrough in the treatment of IS, allowing an improvement of functional outcomes. Nonetheless, the fact that poor outcome occurs in approximately half of the patients with successful thrombectomy[50,51] has stressed the need for complementary therapies. Targeting of platelets and of other components of the early thromboinflammatory cascade triggered by cerebral ischemia has been proposed as a strategy to protect the ischemic microvasculature and penumbral tissue until arterial recanalization. Such strategies are intended to prevent microthrombosis

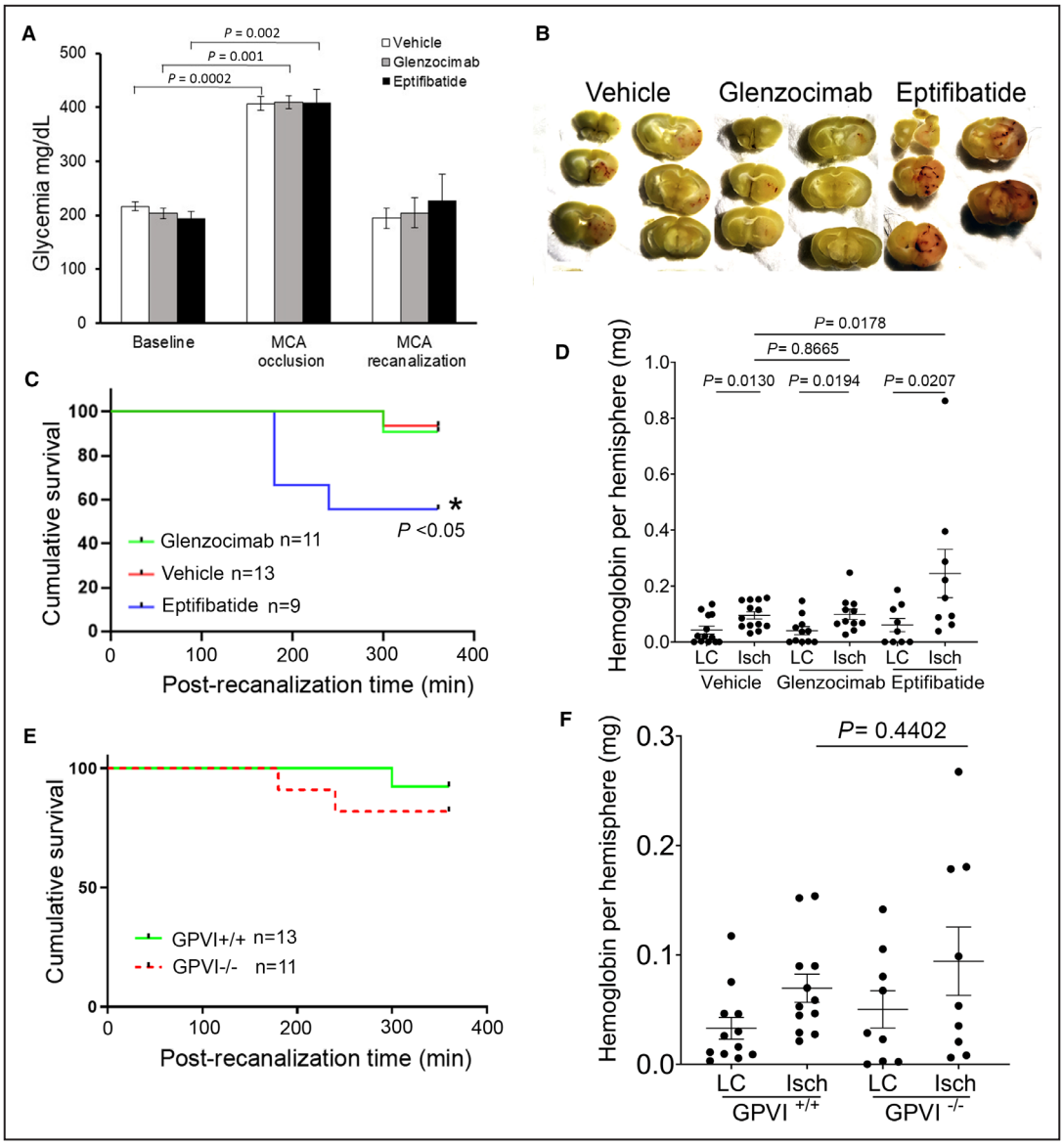

Figure 5. Impact of glenzocimab and eptifibatide in a mouse model of hemorrhagic transformation of ischemic stroke.

A, Glycemia evolution in mice injected with a bolus of glucose (2.2 g glucose/kg IP) 10 min before middle cerebral artery occlusion. n=9–13 mice per group. **B**, Representative images of hemorrhagic transformation of ischemic stroke 6 h after induction of tMCAO under acute hyperglycemia in transgenic mice expressing human GPVI and treated with glenzocimab (64 mg/kg) or eptifibatide (50 mg/kg) administered as a bolus (50% intravenously, 50% intraperitoneally) at recanalization. The treatments were repeated 3 h after recanalization for maintenance. Vehicle corresponds to a mixture of saline with citrate buffer. **C**, Survival curves of mice expressing human GPVI according to treatment in the first 6 h following induction of tMCAO under acute hyperglycemia. **D**, Quantification of hemoglobin content in contralateral and ischemic brain hemispheres of mice mice expressing human GPVI according to treatment. **E**, Survival curves of GPVI$^{+/+}$ and GPVI$^{-/-}$ mice in the first 6 hours following induction of tMCAO under acute hyperglycemia. **F**, Quantification of hemoglobin content in contralateral and ischemic brain hemispheres of GPVI$^{+/+}$ and GPVI$^{-/-}$ mice. Survival curves were plotted by Kaplan–Meier method and differences between survival curves were analyzed by log-rank test. GPVI indicates glycoprotein VI; and tMCAO, transient middle cerebral occlusion.

caused by reciprocal interactions between activated endothelial cells, platelets, neutrophils, and fibrin, which are triggered immediately after arterial occlusion.[1,4,52–55] Remarkably, data from preclinical studies have indicated that antiplatelet strategies have maximal efficacy when given before recanalization.[5,6,15] Yet for legitimate safety concerns as to the risk of worsening bleeding in case of hemorrhagic stroke, in clinical

trials for IS, administration of glenzocimab and eptifibatide has required prior exclusion of ICH and has been performed in combination with standard-of-care recanalization therapy. Our results showing that neither glenzocimab nor GPVI deficiency impact bleeding or death in models of primary or secondary ICH indicate that this concern may deserve reconsideration in the case of drugs targeting GPVI. To our knowledge, this is the first preclinical study demonstrating the safety of an antiplatelet drug in the context of ICH. In addition to expanding available data on the safety profile of glenzocimab and of GPVI targeting, these results bear potentially important clinical implications. If translatable to human IS, as suggested by our humanized mouse model, one could envision the administration of anti-GPVI drugs like glenzocimab upon suspicion of IS, before assessment of its ischemic nature by imaging, thus allowing a considerable acceleration of intervention for maximal neuroprotective benefits. This would be of particular interest for designing trials aimed at determining drug efficacy. In fact, time is of the essence in IS management and this adage also holds true when it comes to GPVI inhibition. GPVI is not only the primary platelet receptor for collagen but also a central mediator of platelet procoagulant activity,[56–59] and it has long been known that blockade of GPVI-dependent platelet activation can be rescued by thrombin.[60] Therefore, GPVI inhibitors might become much less effective in microvascular territories where significant procoagulant activity of platelets has already developed and thrombin formed. Such territories have been shown to expand progressively with the duration of ischemia[1] and, consequently, with the time-to-treatment initiation.

On the other hand, our results confirm that eptifibatide treatment initiation requires prior exclusion of ICH, as eptifibatide increased ICH severity in both models tested. The increased hemorrhagic risk associated with targeting of glycoprotein IIb/IIIa is consistent with its role as the final effector of platelet aggregation common to all platelet activation pathways, which greatly limit the possibility of functional redundancy and compensatory mechanisms for ensuring hemostasis in case of blood vessel rupture.

Our study has several limitations. Although no difference in safety or efficacy has been reported between men and women treated with either glenzocimab[9] or eptifibatide,[61] the exclusive use of male mice in the present study limits the generalizability of our findings across sex. In addition, both of the models we used here were particularly severe and not suitable for accurate longer-term evaluation of outcomes less severe than bleeding or death. It is also worth noting that the doses of glenzocimab and eptifibatide tested in the present safety study largely exceeds the antithrombotic doses used in the clinics or in acute models of thrombosis. One cannot exclude that eptifibatide might prove safer when used at a lower dosage.

In conclusion, in agreement with recent experimental data indicating that new antithrombotic drugs targeting GPVI have a remarkable safety profile from a hemostatic perspective,[7,9,15] our results indicate that glenzocimab remains safe even in the context of ICH. These data could help refine the design of future clinical trials aimed at determining the clinical efficacy of anti-GPVI drugs in IS.

ARTICLE INFORMATION

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Disclosures

Dr Jandrot-Perrus is a scientific founder and scientific adviser of Acticor Biotech, the company that developed glenzocimab. The remaining authors have no disclosures to report.

Supplemental Material

Figures S1–S2

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