ORIGINAL ARTICLE

Rivaroxaban Promotes Reduction of Embolus Size within Cerebrocortical Microvessels in a Mouse Model of Embolic Stroke

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Previous reports have suggested that direct oral anticoagulants exert a prothrombolytic effect against intracardiac thrombi. We hypothesized that these anticoagulants may also help recanalize occluded intracranial arteries via prothrombolytic effects. In this study, we evaluated the effects of rivaroxaban, a direct oral anticoagulant, on fibrin emboli within the cerebrocortical microvessels in a mouse model of embolic stroke. Fibrin emboli prepared *ex vivo* were injected into the common carotid artery of male C57BL/6 mice, and embolization in the microvessels on the brain surface was observed through a cranial window. Oral administration of rivaroxaban was initiated a week before injection of the emboli. The number and sizes of the emboli were measured at two time points: immediately after and 3 h after the embolus injection in the rivaroxaban-treated mice (n =6) and untreated mice (n =7). The rates of recanalization and change in the embolus size were analyzed between the two groups. Complete recanalization was observed only in the rivaroxaban group (three mice in the rivaroxaban group compared with none in the control group). A significantly higher rate of reduction of the embolus size was observed in the rivaroxaban group than in the control group (*P*=0.0216). No significant differences between the two groups were observed in the serum levels of the following coagulation markers: thrombin–antithrombin III complexes, D-dimers, or plasmin–α2-plasmin inhibitor complex. Our findings indicate that rivaroxaban may promote reduction in the size of stagnated fibrin emboli in cerebrocortical microvessels in cases of embolic stroke.   (DOI: 10.2302/kjm.2018-0010-OA)

Keywords: **rivaroxaban, embolic stroke, intracranial embolism, microvessels, prothrombolytic effect, direct oral anticoagulants (DOACs)**

Introduction

Direct oral anticoagulants (DOACs) have recently proven effective for preventing cardioembolic stroke in patients with nonvalvular atrial fibrillation (NVAF).[1] Moreover, DOACs are widely preferred over warfarin potassium. Typically, anticoagulation therapies, including DOACs and warfarin potassium, are used to prevent recurrent cardioembolic stroke.[1,2] However, some reports have suggested that DOACs may also exert a prothrom-

bolytic effect against intracardiac thrombi.[3,4] Inhibition of thrombin activatable fibrinolysis inhibitor (TAFI), which can be activated by thrombin and thrombomodulin, is likely one mechanism underlying the prothrombolytic effect of DOACs.[5] From these findings, we speculated that DOACs, via prothrombolytic effects, may help recanalize intracranial arteries that have been occluded by cardiogenic fibrin emboli.

Several studies have suggested that improved clinical recovery occurs in patients with subacute recanalization

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of the occluded arteries by rescue of the penumbra, an unstable ischemic region around the ischemic focus.[6–9] Nevertheless, initiation of anticoagulant treatment in the acute phase of ischemic stroke is generally not recommended because of the risk of intracranial bleeding events.[10,11] However, DOACs may be administered safely even in the acute phase of ischemic stroke because of the lower risk of bleeding events compared to the risks associated with warfarin potassium.[1,12,13]

Considering the greater safety and possible local prothrombolytic effects of DOACs, we hypothesized that, in cases of acute embolic stroke, early initiation of DOAC administration may promote reduction of embolus size, with only a low risk of intracranial hemorrhagic events. In the current study, we evaluated the effects of rivaroxaban, a DOAC that inhibits factor Xa, on fibrin emboli within the cerebrocortical microvessels in a mouse model of embolic stroke.

Materials and Methods

Animals

The experimental protocol was approved by the Ethics Committee on Animal Care and Use, Keio University (#09058). The male C57BL/6 mice aged 9–11 weeks used in this study were purchased from CLEA Japan Inc. (Tokyo, Japan).

Anesthesia

All animal procedures were performed with the animals under general anesthesia induced by 2.0% isoflurane (Pfizer, New York, NY, USA) and maintained with 1.5%–1.7% isoflurane mixed with room air. No artificial mechanical ventilation was used during the experiments.

Cranial window

A cranial window was created using a previously described method.[14] Briefly, using an operating microscope (S21; Carl Zeiss, Oberkochen, Germany), the cranial bone was drilled and thinned above the left parieto-occipital cortex, 4.0 mm posterior to the bregma and 4.0 mm to the left of the midline, until the brain parenchyma and vessels could be observed through the thinned bone and dura. The diameter of the cranial window was approximately 3.0 mm. After creating the window, we dripped oil on this segment and sealed it with a circular 140-μm-thick quartz coverslip using cement (**Fig. 1A**). A metal bar was attached to the skull of the mouse to enable multiple observations of the same site in the same animal. Microvessels on the brain surface could be observed through the cranial window under a fluorescence microscope equipped with an Hg lamp (LH-M100CB-a; Nikon
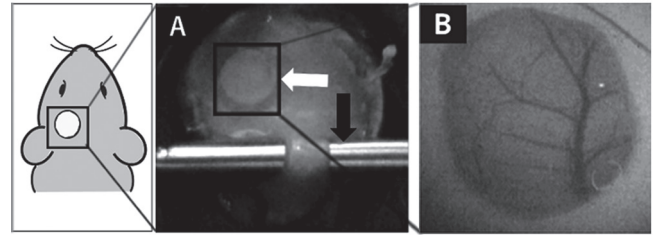

Fig. 1 (A) Cranial window (white arrow) created above the left parieto-occipital cortex, 4 mm posterior to the bregma and 4 mm to the left of the midline. A metal bar (black arrow) was attached to the skull to hold the mouse in position and to enable multiple observations of the same site through the cranial window. (B) The brain parenchyma and vessels could be observed through the cranial window under a bright-field microscope.

Co., Tokyo, Japan) and a band-pass filter (G2A, Nikon Co.) (**Fig. 1B**).

Fibrin emboli

Fibrin emboli were prepared *ex vivo* using a previously reported method with modification.[15] Briefly, 10 mg of human fibrinogen (Sigma-Aldrich Co.; St Louis, MO, USA), 0.2 units of human thrombin (Sigma-Aldrich Co.), and 6 mg of rhodamine B isothiocyanate-dextran 70 (RITC-dextran; 8 mg/ml; Sigma-Aldrich Co.) were mixed with 1 mL of normal saline, and the mixture was refrigerated at 4°C overnight. Before injection of the embolic mixture on the following day, the fibrin emboli were homogenized using 0.5 mL normal saline and then passed through a hydrophilic nylon net filter (pore size, 41.0 μm; Merck Millipore Ltd., Burlington, MA, USA). The fibrin emboli could be visualized under a fluorescence microscope (**Fig. 2**).

Rivaroxaban administration and analysis of blood concentrations

We divided the mice into two groups: the rivaroxaban group and the control group. The mice were housed under a 12-h day/night cycle. Administration of laboratory chow, either mixed or not mixed with rivaroxaban (1200 ppm), was initiated 7 days before injection of the embolic mixture. To confirm the blood concentrations of rivaroxaban, 12 mice (control group, n=6; rivaroxaban group, n=6), in addition to the mice that were used for the embolic mixture injection experiment, were sacrificed 7 days after the initiation of feeding (according to a schedule similar to that in the embolic mixture injection experiment), and blood samples were collected from the heart. The blood samples were centrifuged at 2000 rpm for 5 min to obtain serum samples. The rivaroxaban
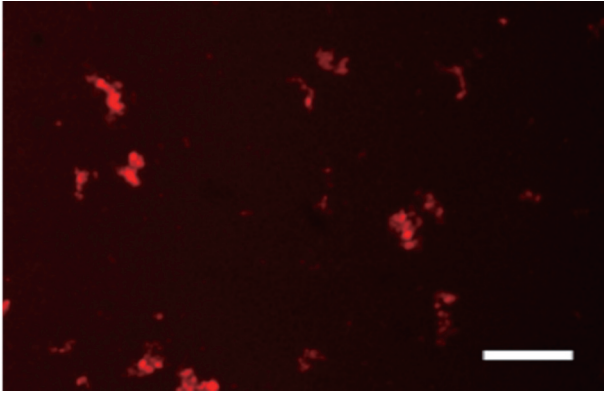
Fig. 2 Image of the embolic mixture before injection. The mixture was prepared *ex vivo* and visualized under a fluorescence microscope. The emboli were prepared by mixing fibrin, fibrinogen, and rhodamine dextran and the mixture was homogenized. The homogenized fibrin-rich embolic mixture was then stored at 4°C until injection on the next day. Scale bar =200 μm.

concentrations in these serum samples were determined using liquid chromatography (Shin Nippon Biomedical Laboratories Ltd., Tokyo, Japan).

Injection of the embolic mixture

The experimental protocol is shown in **Fig. 3**. We used 16 mice (9 mice in the control group and 7 mice in the rivaroxaban group) for this experiment and compared data between 7 mice in the control group and 6 mice in the rivaroxaban group, as described in the "Results" section. The cranial window was created at approximately 10:00 pm on the night before injection of the embolic mixture. Simultaneously, we also prepared the embolic mixture by mixing fibrinogen, thrombin, and rhodamine dextran and kept the mixture under refrigeration. At 8:00–9:00 am on the following morning, the mice were anesthetized and placed in the supine position. A catheter was then inserted into the left common carotid artery (CCA). The animals were subsequently placed in the prone position so that the brain surface could be microscopically observed through the cranial window. We then injected 0.1 mL of homogenized embolic mixture through the catheter and confirmed the presence of stagnated emboli in the arteries on the brain surface by observation with a fluorescence microscope through the cranial window. If stagnated emboli were not identified after the first injection, further 0.05-mL injections (up to a maximum of four additional injections) were administered until stagnated emboli were seen. After confirmation of emboli, we removed the catheter, closed the skin incision, and left the mice in their home cages with free access to water and food. Three hours later, the mice were anesthetized again
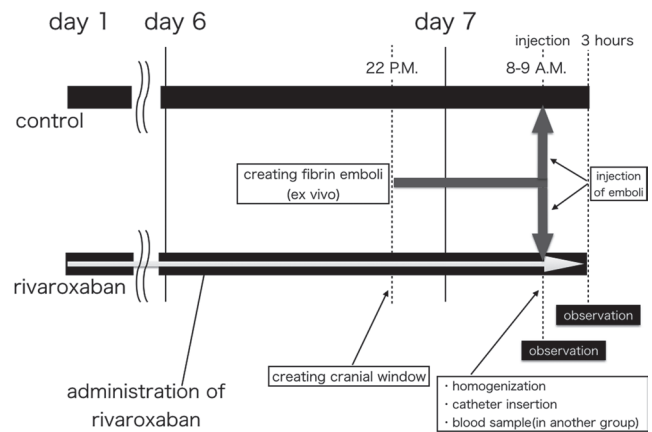

Fig. 3 Schematic of the experimental protocol. Administration of laboratory chow mixed or not mixed with rivaroxaban (1200 ppm) was started a week before the injection of the embolic mixture. At approximately 10:00 pm on the 6th day, the cranial window was created, and the fibrin-rich embolic mixture was prepared *ex vivo*. On the following morning (8:00–9:00 am), the embolic mixture was homogenized, a catheter was inserted into the left common carotid artery (CCA), and the fibrin-rich embolic mixture was injected into the left CCA. We measured the number and sizes of the emboli formed immediately after the injection and 3 h later. Blood sampling was performed in another group of mice by following the same schedule.

and held in place by the metal bar so that the same site of the brain surface could be observed once again through the cranial window.

Image analysis

Images observed through the cranial window were captured by a video camera attached to the fluorescence microscope at two time points: immediately after and 3 h after injection of the embolic mixture. Emboli labeled with rhodamine were detected under an Hg lamp, and the other nonfluorescent structures were evident in the bright-field images.

The total number of emboli observed through the cranial window immediately after injection of the emboli mixture was counted by three examiners who were blinded to the groups to which the animals had been allocated (rivaroxaban/control). The number of emboli that were still observable in the same locations 3 h after injection of the emboli mixture was also counted by the three blinded examiners. The rate of recanalization was calculated according to the following formula:

$$Recanalization\ rate\,(\%)$$
$$= \frac{number\ of\ emboli\ immediately\ after\ injection - number\ of\ emboli\,3\ hours\ later}{number\ of\ emboli\ immediately\ after\ injection}$$
$$\times 100.$$

Additionally, we measured the sizes of the emboli at each time point in each animal using Image J,[16] which is free calculation software. The sum of the sizes of all the emboli visualized through the cranial window was measured by three blinded examiners. The sum of the sizes of the emboli th were still observable at the same locations 3 h after injection of emboli was again measured by the three examiners. The rate of change of the embolus size was calculated according to the following formula:

$$Rate\ of\ change\ in\ embolus\ size\,(\%)$$
$$= \frac{the\ sum\ of\ the\ embolus\ size\,3\ hours\ after\ the\ injection\,(pixels)}{the\ sum\ of\ the\ embolus\ size\ immediately\ after\ the\ injection\,(pixels)}$$
$$\times 100.$$

The median value of the data counted/measured by the three examiners was adopted for all image analyses.

Measurements of coagulation markers in blood

We obtained serum samples from 10 mice that were not part of the injected thrombi experiments (control group, n=5; rivaroxaban group, n=5) to measure the blood levels of coagulation and thrombolysis markers, i.e., thrombin–antithrombin III complex (TAT), D-dimers, and plasmin–α2-plasmin inhibitor complex (PIC). To reproduce the conditions, all procedures performed on the mice in the embolic mixture injection experiment were also performed on this group. The mice were sacrificed 3 h after injection of the embolic mixture. Analysis of the blood samples was outsourced to SRL Inc., Tokyo, Japan.

Statistical analysis

The Mann–Whitney U test was used to compare data between the groups. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using GraphPad PRISM version 6 (GraphPad Software, Inc. CA, USA).

Results

Blood concentrations of rivaroxaban

After administration of rivaroxaban for 7 days, the blood concentrations of rivaroxaban in 6 mice (the rivaroxaban group) were in the range of 0.292–1.25 µg/ml (mean, 0.662 µg/ml), which closely matched the adequate range, 0.4–1.0 µg/ml in daylight, as previously determined by Bayer Pharma AG, a manufacturer of rivaroxaban. (This

adequate range was obtained using animals administered with a chow diet containing 1200 ppm rivaroxaban; this drug concentration was determined to inhibit clot formation by >70% in the rodent venous thromboembolism model).[17] Conversely, the blood level of rivaroxaban was below the detection limit in the control group (n=6) fed with normal laboratory chow for 7 days.

Observation of injected emboli through the cranial window

In the current experiment, there was possibly a difference in the duration of laser exposure between the two groups, and this difference could potentially influence the result through reduced intensity of the fluorescence of emboli. However, in an *ex vivo* study, we confirmed that the size of emboli did not change under continuous laser exposure for 5 min (data not shown); the total observation time under a fluorescence microscope for the two time points in the current experiment was typically less than 2 min.

Before they were injected, the diameters of the emboli were measured under a fluorescence microscope, and the mean ± SD was 13.2 ± 5.0 µm. A recently published article from our group stated that artificial microspheres with a diameter ranging from 13 to 24 µm could distribute through the arteries in the cortex.[18] Consequently, the size of the emboli prepared using our method was considered suitable for observing emboli in the microvessels on the brain surface.

After injection via the CCA, the embolic mixture labeled with rhodamine traveled through the arteries and finally coalesced and stagnated in the microvessels on the brain surface. The resulting stagnated emboli were successfully visualized through the cranial window using a camera attached to the fluorescence microscope (**Fig. 4A–H**, a supplementary video is also available).

Calculation of the recanalization rates and rates of change in embolus size

Another batch of mice was included in this study (nine mice in the control group and seven mice in the rivaroxaban group). One mouse in the control group and one mouse in the rivaroxaban group died during the interval between injection of the embolic mixture and the observation performed 3 h after the injection. Another mouse in the control group was excluded because no stagnated emboli could be observed through the cranial window, even after four additional embolus injections. Therefore, seven mice in the control group and six mice in the rivaroxaban group finally underwent calculation of the recanalization rate and the rate of change in emboli size. [In most cases, the stagnated emboli could be visualized after a single injection (0.1 mL) of the embolic mixture.
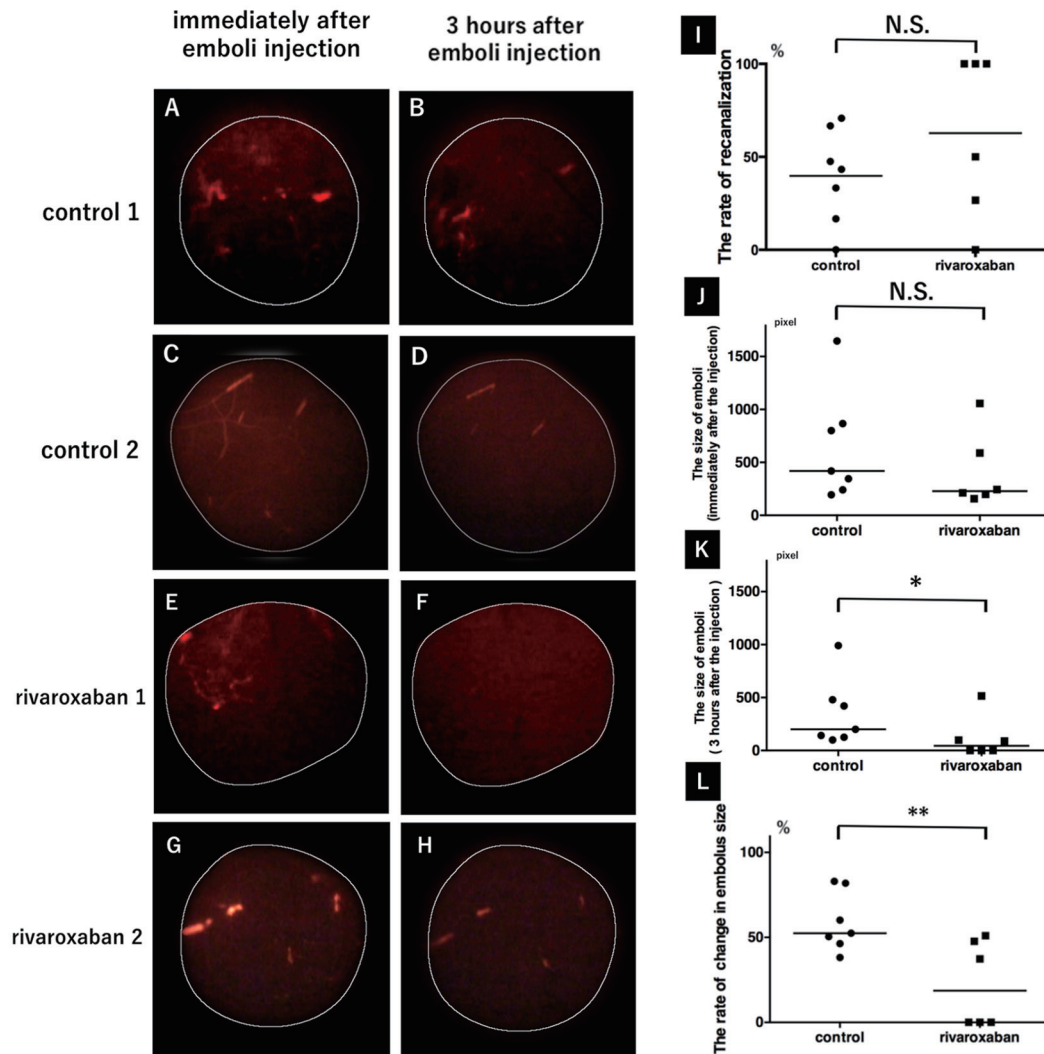
Fig. 4 (A–D) Representative images of the stagnated emboli within the intracranial arteries visualized under a fluorescence microscope in the control group [(A,C) immediately after injection of emboli and (B,D) 3 h after emboli injection] and (E–H) in the rivaroxaban group [(E,G) immediately after emboli injection and (F,H) 3 h after injection of emboli]. The white circle outlines indicate the areas observed through the cranial windows. (I) There were no significant differences in the rate of recanalization between the control group (n =7) and the rivaroxaban group (n =6). However, mice with complete (100%) recanalization were observed only in the rivaroxaban group (3 of 6 mice), and no mice with complete recanalization were observed in the control group (0 of 7 mice). (J,K) Comparison of the size of emboli between the two groups. (J) The embolus size immediately after injection of the embolic mixture did not significantly differ between the two groups, whereas, (K) at 3 h after the injection, the embolus size was significantly smaller in the rivaroxaban group than in the control group (*P=0.0344). (L) Calculation of the rate of change of the embolus size revealed a significantly higher rate of decrease in the embolus size in the rivaroxaban group than that in the control group (**P=0.0216). N.S., no significant difference.

However, three mice in the rivaroxaban group and three mice in the control group required a single additional injection (0.05 mL of the mixture) for successful visualization of emboli].

At 3 h after emboli were injected, new emboli were occasionally observed at sites where emboli were not observed immediately after the injection of emboli. We speculated that these emboli were located at the proximal parts of arteries that were initially outside the field of view through cranial windows. These emboli then traveled to the area where they could be observed through the cranial window 3 h after injection. However, it was difficult to confirm this hypothesis. Moreover, it was impossible to calculate the chronological change in each embolus when we included these newly observed emboli in the analysis. Therefore, we focused only on the emboli

that were still visible in the same location at 3 h after injection of emboli.

The median (interquartile range) of the recanalization rate in the control group and rivaroxaban group were 43.3% (16.7%–66.7%) and 75.0% (20.0%–100.0%), respectively (**Fig. 4I**). This difference was not significant between the groups (P=0.3077). However, mice with complete (100%) recanalization were observed only in the rivaroxaban group (three of six mice), and no mice with complete recanalization were observed in the control group (none of seven mice).

In the detailed observation of emboli, some did not disappear but had decreased in size by 3 h after the injection (representative images are shown in **Fig. 4C,D**). This change was recognized as "partial recanalization at each occluded site" and is important in clinical situations. However, the analysis of the recanalization rate did not reflect these partial changes. Therefore, we proceeded to analyze the embolus size. First, the embolus size immediately after injection did not differ between the two groups (P=0.3566; **Fig. 4J**). This finding confirmed that the fibrin emboli prepared *ex vivo* were not influenced by administration of rivaroxaban prior to the emboli injection. At 3 h after injection of the embolic mixture, the total embolus size was significantly smaller in the rivaroxaban group than in the control group (P=0.0344, **Fig. 4K**). Moreover, the rivaroxaban group had a significantly higher rate of decrease of the embolus size than the control group did (P=0.0216). The median (interquartile range) rates of change in the control group and rivaroxaban group were 52.5% (46.3%–81.9%) and 18.7% (0.0%–48.5%), respectively. These data suggested that rivaroxaban promoted the size reduction of emboli within cerebrocortical microvessels.

Analysis of coagulation markers in blood

To investigate the mechanism underlying the decrease in embolus size in the rivaroxaban group, the blood levels of coagulation markers TAT, PIC, and D-dimers were measured. The levels of TAT in the control group and rivaroxaban group were not significantly different (2.8 ± 1.7 vs. 2.3 ± 1.7 ng/ml; mean ±SD), whereas the levels of PIC and D-dimers were below the respective detection limits in both groups.

Discussion

We successfully observed stagnated fibrin emboli in the cerebral microvessels on the brain surface through a cranial window created in a mouse model of embolic stroke after injection of an embolic mixture into the CCA. Furthermore, the rate of reduction of the embolus size was significantly higher in the rivaroxaban group than in the control group.

Several studies have described a mouse model of embolic stroke using fibrin-rich clots labeled with fluorescents.[15,19,20] We combined this embolic stroke model with the creation of a cranial window that allowed direct visualization of the stagnated fibrin emboli in the cerebral microvessels on the brain surface. As a result, our model could provide *in vivo* imaging and multiple chronological observations of the same area on the brain surface in the same animal, which is useful, for example, to investigate the effects of drugs *in vivo*.

We propose the following two mechanisms to explain the decrease in embolus size in the rivaroxaban group. The first is that rivaroxaban inhibits secondary thrombus formation by exerting an anticoagulant effect. This effect results in the relative dominance of the endogenic thrombolytic mechanism, which ultimately leads to earlier lysis of thrombus. The second is that rivaroxaban itself exerts a prothrombolytic effect.

Because rivaroxaban exerts its anticoagulant effect via inhibition of thrombin activation, direct measurement of thrombin activation would be helpful to confirm the anticoagulation state and secondary thrombus inhibition induced by rivaroxaban (**Fig. 5**). However, direct measurement of thrombin activation is impossible because of the short half-life of thrombin. Activated protein C is another candidate molecule to verify the inhibition of secondary thrombus formation. Although protein C production is preserved under treatment with rivaroxaban, activation of protein C is attenuated by a decrease in thrombin–thrombomodulin complex caused by rivaroxaban-induced inhibition of thrombin formation. However, we decided to measure the blood levels of TAT as a coagulation marker because a previous study reported that rivaroxaban inhibited TAT in a dose-dependent manner.[21]

In the present study, the blood TAT levels were not significantly different between the two groups and were within the normal range (<3 ng/ml) in both groups. A plausible reason for this finding is that the tiny emboli and focal thrombotic reaction in this experiment were insufficient to influence the levels of the coagulation marker, which were measured in systemic circulating blood samples. However, in clinical settings, it is not necessary to monitor the blood levels of coagulation markers in patients treated with DOACs. Moreover, it has been shown that a fixed dose of DOACs is effective as prophylaxis against cardiogenic embolic stroke in patients with NVAF.[12] In the present study, the plasma concentration of rivaroxaban was in the adequate range in the rivaroxaban group; therefore, we considered the anticoagulant effect of rivaroxaban as guaranteed, even in the absence of monitoring of coagulation markers.

To consider the prothrombolytic effect of anticoagulants, the measurement of TAFI activation is important. TAFI is a plasma procarboxypeptidase that can be activated by thrombin and the thrombomodulin complex, and activated TAFI inhibits thrombolysis (**Fig. 5A**).[22] A pre-
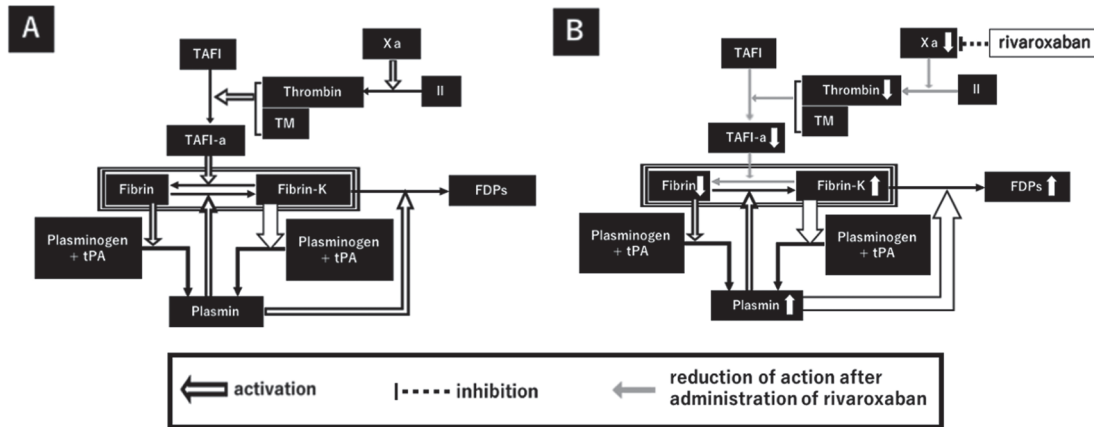
Fig. 5 Schematic of the thrombolytic process. Fibrin works as a cofactor promoting the binding of t-PA to plasminogen, which results in increased formation of plasmin and enhanced thrombolysis. The C-terminal lysine of fibrin is exposed by the effect of plasmin, and this exposed type of fibrin (Fibrin-K) promotes more efficient binding of t-PA to plasminogen than does the common type of fibrin, which results in further enhancement of thrombolysis. (A) TM and the thrombin complex activate TAFI, and activated TAFI removes the C-terminal lysine of fibrin, which results in inhibition of thrombolysis through the mechanism mentioned above. (B) Rivaroxaban inhibits factor Xa, reduces the generation of thrombin, and inhibits activation of TAFI, which leads to abrogation of the activated TAFI-induced inhibition of thrombolysis. TM, thrombomodulin; TAFI, thrombin activatable fibrinolysis inhibitor; Xa, factor Xa; tPA, tissue-type plasminogen activator; FDPs, Fibrin/fibrinogen degradation products.

vious report suggested that rivaroxaban reduces thrombin generation by inhibiting factor Xa, which potentially results in reduced TAFI activation and leads to thrombolysis (**Fig. 5B**).[5] However, because no assay for TAFI is currently available in domestic companies, we could not verify this mechanism in our model. Instead, we measured two markers of thrombolysis, PIC and D-dimers, in the blood. However, the levels of both markers were below the limit of detection in both groups, and we could not verify the mechanisms underlying the prothrombolytic effect of rivaroxaban.

Taken together, the results of our study suggest that early initiation of DOACs may improve the microcirculation in the cerebral cortex by reducing the size of fibrin emboli. Although a prothrombolytic effect of rivaroxaban was not verified in our study, a relatively dominant endogenous thrombolytic effect, which resulted in the inhibition of secondary thrombus formation by exerting an anticoagulant effect, was considered to be the likely mechanism of the results obtained in our study. A study to compare factor Xa inhibitors, such as rivaroxaban, and direct thrombin inhibitors, such as dabigatran, would be another way to investigate the mechanism of embolus size reduction. However, administration of dabigatran to animals is currently impossible because the drug remains stable only within capsules.

There were three important limitations to our study. First, to consider the clinical application of our results to stroke therapy, it would have been more rational to administer rivaroxaban after the injection of the fibrin emboli. However, in our study protocol, rivaroxaban was designed to be administered prior to the injection of the embolic mixture to obtain the adequate range of the drug and because of the practical difficulty of administering rivaroxaban to animals. However, because the fibrin-rich embolic mixture was prepared *ex vivo* and did not contain any material from the animal treated with rivaroxaban, prior administration of rivaroxaban to animals would not be expected to have any significant effect on the fibrin emboli observed immediately after injection of the embolic mixture. Furthermore, we must consider an important difference between the clinical situation and our animal study: in the clinical situation in which drugs are administrated after the onset of stroke, time is required to obtain an adequate concentration of drugs. However, in our study, the concentration of rivaroxaban was already in the adequate range when the embolic mixture was injected. Second, because we used rhodamine dextran to enable observation of the microemboli, exposure to light and laser radiation could have reduced the intensity of fluorescence and caused a resultant apparent decrease in the embolus sizes as observed through the fluorescence microscope. To resolve this problem, we attempted to avoid unnecessary exposure to light as much as possible. Third, we homogenized the fibrin and used a filter to exclude emboli that were >40 μm in diameter, although the emboli were not uniform in size. This could have influenced the distribution of the embolus sizes in the two

groups of mice. Nevertheless, we found no significant difference between the two groups in the embolus size immediately after injection, which suggests the absence of any significant effect of variation in the sizes of the emboli on the results of comparison between the two groups. Furthermore, our model is not suitable for comparison of infarct sizes because the size of the injected emboli used in this model was too small to cause infarction.

Conclusion

In the current study, we investigated the effect of rivaroxaban on fibrin emboli in cerebrocortical microvessels by direct observation through the creation of a cranial window in a mouse model of embolic stroke. The rate of reduction in embolus size was significantly higher in the rivaroxaban group than in the control group. This result suggests that, rather than acting as prophylaxis against embolic stroke, the administration of rivaroxaban, even after embolic stroke has occurred, might be effective in inducing a reduction of embolus size in the intracranial arteries. Although the underlying mechanism remains unclear, we believe that early initiation of DOACs in embolic stroke patients might lead to earlier lysis of emboli relative to that in patients not receiving DOACs. Additional basic and clinical research is required to determine whether early initiation of DOACs leads to a better clinical outcome.

Acknowledgements

Supplementary video legend

The supplementary video shows the rhodamine-labeled fibrin emboli visualized through the cranial window. A fibrin-rich embolic mixture was injected into the left common carotid artery, which resulted in stagnation of the fibrin thrombi within the intracranial arteries on the brain surface.

References

1. Ntaios G, Papavasileiou V, Diener HC, Makaritsis K, Michel P: Nonvitamin-K-antagonist oral anticoagulants in patients with atrial fibrillation and previous stroke or transient ischemic attack: a systematic review and meta-analysis of randomized controlled trials. Stroke 2012; **43**: 3298–3304. PMID:23150654, DOI:10.1161/STROKEAHA.112.673558
2. EAFT (European Atrial Fibrillation Trial) Study Group: Secondary prevention in non-rheumatic atrial fibrillation after transient ischaemic attack or minor stroke. Lancet 1993; **342**: 1255–1262. PMID:7901582
3. Takasugi J, Yamagami H, Okata T, Toyoda K, Nagatsuka K: Dissolution of the left atrial appendage thrombus with rivaroxaban therapy. Cerebrovasc Dis 2013; **36**: 322–323. PMID:24192983, DOI:10.1159/000354315
4. Kawakami T, Kobayakawa H, Ohno H, Tanaka N, Ishihara H: Resolution of left atrial appendage thrombus with apixaban. Thromb J 2013; **11**: 26. PMID:24359320, DOI:10.1186/1477-9560-11-26
5. Komori M, Yasaka M, Kokuba K, Matsuoka H, Fujimoto S, Yoshida M, Kameda K, Shono T, Nagata S, Ago T, Kitazono T, Okada Y: Intracranial hemorrhage during dabigatran treatment. Circ J 2014; **78**: 1335–1341. PMID:24662438, DOI:10.1253/circj.CJ-13-1534
6. Qureshi AI, Hussein HM, Abdelmoula M, Georgiadis AL, Janjua N: Subacute recanalization and reocclusion in patients with acute ischemic stroke following endovascular treatment. Neurocrit Care 2009; **10**: 195–203. PMID:19051064, DOI:10.1007/s12028-008-9161-0
7. Wunderlich MT, Goertler M, Postert T, Schmitt E, Seidel G, Gahn G, Samii C, Stolz E, Duplex Sonography in Acute Stroke (DIAS) Study Group Competence Network Stroke: Recanalization after intravenous thrombolysis: does a recanalization time window exist? Neurology 2007; **68**: 1364–1368. PMID:17452580, DOI:10.1212/01.wnl.0000260604.26469.8e
8. von Kummer R, Holle R, Rosin L, Forsting M, Hacke W: Does arterial recanalization improve outcome in carotid territory stroke? Stroke 1995; **26**: 581–587. PMID:7709401, DOI:10.1161/01.STR.26.4.581
9. Iguchi Y, Kimura K, Hayashi K, Shibazaki K, Kobayashi K, Inoue T: Spontaneous recanalization at subacute phase of stroke may be dramatic: a case report. J Neurol Sci 2007; **252**: 92–95. PMID:17125795, DOI:10.1016/j.jns.2006.10.002
10. International Stroke Trial Collaborative Group: The International Stroke Trial (IST): a randomised trial of aspirin, subcutaneous heparin, both, or neither among 19 435 patients with acute ischaemic stroke. Lancet 1997; **349**: 1569–1581. PMID:9174558, DOI:10.1016/S0140-6736(97)04011-7
11. Paciaroni M, Agnelli G, Micheli S, Caso V: Efficacy and safety of anticoagulant treatment in acute cardioembolic stroke: a meta-analysis of randomized controlled trials. Stroke 2007; **38**: 423–430. PMID:17204681, DOI:10.1161/01.STR.0000254600.92975.1f
12. Patel MR, Mahaffey KW, Garg J, Pan G, Singer DE, Hacke W, Breithardt G, Halperin JL, Hankey GJ, Piccini JP, Becker RC, Nessel CC, Paolini JF, Berkowitz SD, Fox KA, Califf RM, ROCKET AF Investigators: Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. N Engl J Med 2011; **365**: 883–891. PMID:21830957, DOI:10.1056/NEJMoa1009638
13. Seiffge DJ, Traenka C, Polymeris A, Hert L, Peters N, Lyrer P, Engelter ST, Bonati LH, De Marchis GM: Early start of DOAC after ischemic stroke. Neurology 2016; **87**: 1856–1862. PMID:27694266, DOI:10.1212/WNL.0000000000003283

14. Tomita Y, Kubis N, Calando Y, Dinh AT, Méric P, Seylaz J, Pinard E: Long-term in vivo investigation of mouse cerebral microcirculation by fluorescence confocal microscopy in the area of focal ischemia. J Cereb Blood Flow Metab 2005; **25**: 858–867. PMID:15758950, DOI:10.1038/sj.jcbfm.9600077

15. Atochin DN, Murciano JC, Gürsoy-Özdemir Y, Krasik T, Noda F, Ayata C, Dunn AK, Moskowitz MA, Huang PL, Muzykantov VR: Mouse model of microembolic stroke and reperfusion. Stroke 2004; **35**: 2177–2182. PMID:15256680, DOI:10.1161/01.STR.0000137412.35700.0e

16. Schneider CA, Rasband WS, Eliceiri KW: NIH Image to ImageJ: 25 years of image analysis. Nat Methods 2012; **9**: 671–675. PMID:22930834, DOI:10.1038/nmeth.2089

17. Morihara R, Yamashita T, Kono S, Shang J, Nakano Y, Sato K, Hishikawa N, Ohta Y, Heitmeier S, Perzborn E, Abe K: Reduction of intracerebral hemorrhage by rivaroxaban after tPA thrombolysis is associated with downregulation of PAR-1 and PAR-2. J Neurosci Res 2017; **95**: 1818–1828. PMID:28035779, DOI:10.1002/jnr.24013

18. Tsukada N, Katsumata M, Oki K, Minami K, Abe T, Takahashi S, Itoh Y, Suzuki N: Diameter of fluorescent microspheres determines their distribution throughout the cortical watershed area in mice. Brain Res 2018; **1679**: 109–115. PMID:29203170

19. Lam CK, Yoo T, Hiner B, Liu Z, Grutzendler J: Embolus extravasation is an alternative mechanism for cerebral microvascular recanalization. Nature 2010; **465**: 478–482. PMID:20505729, DOI:10.1038/nature09001

20. Grutzendler J, Murikinati S, Hiner B, Ji L, Lam CK, Yoo T, Gupta S, Hafler BP, Adelman RA, Yuan P, Rodriguez G: Angiophagy prevents early embolus washout but recanalizes microvessels through embolus extravasation. Sci Transl Med 2014; **6**: 226ra31. PMID:24598589, DOI:10.1126/scitranslmed.3006585

21. Perzborn E, Heitmeier S, Buetehorn U, Laux V: Direct thrombin inhibitors, but not the direct factor Xa inhibitor rivaroxaban, increase tissue factor-induced hypercoagulability *in vitro* and *in vivo*. J Thromb Haemost 2014; **12**: 1054–1065. PMID:24766850, DOI:10.1111/jth.12591

22. Bajzar L: Thrombin activatable fibrinolysis inhibitor and an antifibrinolytic pathway. Arterioscler Thromb Vasc Biol 2000; **20**: 2511–2518. PMID:11116046, DOI:10.1161/01.ATV.20.12.2511