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Letter to the Editors-in-Chief

Novel antithrombotic effects of dabigatran in patients with non-valvular atrial fibrillation

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1. Introductions

Ischemic heart disease and stroke are major causes of death and morbidity worldwide. Coronary and cerebrovascular events are a consequence of thrombus formation caused by atherosclerotic plaque rupture or embolism, both of which result from platelet activation and aggregation and thrombin-mediated fibrin generation via the coagulation cascade [1]. In addition, thrombin is also one of the most effective platelet activators [2]. We focused on a novel thrombus formation mechanism called thrombin-mediated platelet stimulation and established thrombin-induced platelet aggregation [3].

Direct oral anticoagulants (DOACs) have recently come into widespread use as an alternative to warfarin in preventing ischemic stroke in patients with non-valvular atrial fibrillation (NVAF) [4,5]. Dabigatran (Boehringer Ingelheim, Ingelheim, Germany) is a direct and selective thrombin inhibitor considered to have an antiplatelet effect in addition to its anticoagulation effects [6]. As such, it is primarily used for the prevention of stroke in patients with NVAF, and can be expected to inhibit both platelet activation and coagulation [1,3,5,6]. A standard for thrombin-induced platelet aggregation was developed using the CS2400 (Sysmex, Kobe, Japan) in healthy subjects, and dabigatran was confirmed to inhibit thrombin-induced platelet aggregation in vitro with platelet-rich plasma (PRP) [3].

The RE-LY study [7] reported that individual benefit-risk calculations might be improved by tailoring dabigatran doses after considering selected patient characteristics. That is, testing thrombin-induced platelet aggregation before and after drug administration is necessary when evaluating the efficacy of dabigatran.

A previous study investigating the efficacy and safety of DOACs in a Japanese population reported that dabigatran had the effect of preventing atherothrombotic stroke, presumably due to an antiplatelet effect [5]. Therefore, we evaluated whether dabigatran was useful as an antiplatelet drug in patients with NVAF using thrombin-induced platelet aggregation with automated light transmission aggregometry (LTA) [8].

2. Subjects and methods

Between April 2017 and April 2018, 17 consecutive patients (age range, 61–91 years; median age, 78 years; 10 men and 7 women) with NVAF who had not previously received treatment with anticoagulants were prospectively enrolled in a study to investigate the efficacy of dabigatran. This study was approved by the institutional ethics committee (2015–2020). As normal controls, 59 healthy volunteers (17 men, 42 women; mean age, 33 ± 9 years) were recruited from the Iwate Medical University Hospital; all gave written informed consent.

2.1. Sample preparation and analysis

Dabigatran was administered at 110 mg or 150 mg in twice-daily doses to patients with NVAF. Blood samples were collected prior to dabigatran treatment, two weeks after treatment, and four weeks after treatment. Samples were collected from the cephalic vein using a 21-gauge needle at about 11 AM, 4 h after patients were given dabigatran at the first dose of the day, in a tube with 3.13% sodium citrate for aggregation testing. PRP was separated by low-speed centrifugation and platelet-poor plasma (PPP) was obtained by high-speed centrifugation according to standard protocol [9].

Thrombin (Sigma Aldrich. Co., LLC; St Louis, MO, USA) was used to induce platelet aggregation, and aggregometry was performed under stirring conditions in PRP-supplement with a final Gly-Pro-Arg-Pro (GPRP) (Zedira GmbH; Darmstadt, Germany) concentration of 2 mM, following addition of a final concentration of 0.1–1.0 U/mL of thrombin as per Shimizu et al. [10]. Maximum platelet aggregation percent (MA %) was determined by measuring the reaction for 420 s after thrombin addition. Aggregation induced by thrombin receptor-activating peptide (TRAP; BACHEM; Bubendorf, Switzerland) at the range of 0.1–10.0 μM was similarly measured without GPRP reagent [10]

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Table 1
Characteristics of patients with non-valvular atrial fibrillation (NVAF).

Patient characteristic	Number of patients or value
Age (median)	61–91 (78)
Sex	
Male	10 (58.9%)
Female	7 (41.1%)
Acute ischemic stroke	6 (35.3%)
Dabigatran dose	
2 × 110 [mg]	13 (76.5%)
2 × 150 [mg]	4 (23.5%)
CHA_2D_2-VASc (median)	1–6 (4)
Risk factors	
Hypertension	10 (58.9%)
Hyperlipidemia	4 (23.5%)
Diabetes mellitus	4 (23.5%)
Heart failure	2 (11.8%)
Myocardial infarction	1 (5.9%)
Smoking	2 (11.8%)
Creatinine [mg/dL] (median)	0.43–1.10 (0.84)
CCr [mL/min] (median)	35–112 (61.50)
Medication	
Antiplatelet agent	6 (35.3%)
Anticoagulant agent	0 (0.0%)
Calcium channel blocker	7 (41.4%)
ARB & ACE inhibitor	3 (17.6%)
Beta-blocker	1 (5.9%)
Statin	4 (23.5%)
Oral hypoglycemic medication	1 (5.9%)
Insulin	0 (0%)

The results represent the number (percent of prevalence; %). CCr, creatinine clearance; ARB, angiotensin II receptor blocker; ACE, angiotensin-converting enzyme inhibitor; parentheses, median or percent.

2.2. Statistical analysis

All data were analyzed using SPSS version 25 software (IBM Japan, Ltd., Tokyo, Japan). The differences in thrombin-induced platelet aggregation and TRAP-induced platelet aggregation were compared between samples collected before and after taking dabigatran using the Friedman test. In addition, the Mann-Whitney test was used to compare thrombin-induced platelet aggregation between patients treated with and without anti-platelet agents. Differences with $p < .05$ were considered significant.

3. Results

A total of 17 patients with NVAF were enrolled and completed the follow-up examination. Six patients received antiplatelet therapy before dabigatran treatment, with three taking clopidogrel, two taking aspirin, and one taking cilostazol, but all switched to dabigatran alone after dabigatran treatment. Patient details are shown in Table 1.

Thrombin-induced platelet aggregation in NVAF patients was examined in the range of 0.1–1.0 U/mL of thrombin pre-treatment, two weeks after, and four weeks after dabigatran treatment (Fig. 1 (A)). Prior to treatment, thrombin-induced platelet aggregation was elevated in a concentration-dependent fashion. The median MA % and 25th–75th percentile values at 0.1, 0.2, 0.5, and 1.0 U/mL thrombin were 0% (0–0%), 0% (0–0%), 80.3% (71.4–87.7%), and 88.7% (86.2–91.3%), respectively. In the control group, the median MA % and 25th–75th percentile values at 0.1 U/mL, 0.2 U/mL, 0.5 U/mL, and 1.0 U/mL thrombin were 0.0% (0.0–0.0%), 0.0% (0.0–0.0%), 88.0% (84.0–90.3%), and 91.6% (87.6–93.7%), respectively. There was no difference in thrombin-induced platelet aggregation between normal controls and pre-treatment in NVAF patients (Mann-Whitney test). However, thrombin-induced platelet aggregation at two and four weeks after taking dabigatran was almost completely inhibited, even at the highest concentration of thrombin (1.0 U/mL). The median MA % and 25th–75th percentile values two weeks and four weeks after taking dabigatran with 0.1, 0.2, 0.5, and 1.0 U/mL of thrombin were 0.1% (0.0–0.4%) and 0.4% (0.0–2.1%); 0% (0.0–0.2%) and 0% (0.0–0.2%); 0% (0.0–0.0%) and 0% (0.0–0.0%); and 0% (0.0–0.0%) and 0% (0.0–0.0%), respectively. Platelet aggregation at 0.5 and 1.0 U/mL of thrombin was significantly inhibited after dabigatran treatment compared with before ($p < .001$ and 0.001, respectively, Friedman test). However, there was no difference at 0.2 U/mL because of an insufficient concentration of thrombin that could induce platelet aggregation ($p = .119$, Friedman test). In addition, there were no differences between 110 mg and 150 mg at each concentration of thrombin ($p = .193$, Mann-Whitney test). Furthermore, platelet aggregation induced by thrombin was no different between patients with and without anti-platelet agents at each concentration of thrombin ($p = .20$, Mann-Whitney test).

TRAP-induced platelet aggregation was examined in the range of 0.1 to 10.0 μM TRAP at the same three time points (Fig. 1 (B)). These platelet aggregation values were elevated in a concentration-dependent manner in NVAF patients taking dabigatran. The median MA % and 25th–75th percentile values at pre-treatment, two weeks after, and four weeks after taking dabigatran at 0.1, 0.5, 5.0, and 10.0 μM of TRAP were 5.1% (0.2–8.9%), 5.1% (1.5–23.7%), and 4.1% (0.4–17.4%); 8.1% (5.6–19.5%), 12.1% (3.3–21.0%), and 6.7% (0.0–19.9%); 88.6% (86.8–90.0%), 88.6% (87.3–91.1%), and 87.0% (85.9–90.8%); and 87.1% (83.9–90.0%), 86.7% (85.3–90.1%), and 85.2% (82.7–88.9%), respectively. TRAP-induced platelet aggregation was not significantly different at each TRAP concentration at any point in time ($p = .26$, Friedman test). In normal controls, the median MA % and 25th–75th percentile values at 0.1, 0.5, 5.0 μM and 1.0 μM TRAP in normal controls were 0.4% (0.2–1.4%), 0.0% (0–3.75%), 90.3% (87.0–93.5%), and 89.6% (85.8–91.0%), respectively. TRAP-induced platelet aggregation at low doses (0.1 and 0.5 μM) yielded significantly higher MA % in pretreatment NVAF patients compared to normal controls ($p < .05$ and $p < .01$, respectively, Mann-Whitney test). However, there was no difference in TRAP-induced platelet aggregation at 5 and 10 μM between normal controls and before treatment in NVAF patients (Mann-Whitney test).

4. Discussion

The antiplatelet effect of a dabigatran in patients with NVAF was successfully detected using a CS2400 LTA to measure thrombin-induced aggregation using PRP by adding GPRP to inhibit fibrin polymerization. This is the first report describing the detection of dabigatran's antiplatelet effect in blood samples from patients with NVAF in vivo. Although our results indicate that dabigatran has a strong inhibitory effect on thrombin-induced platelet aggregation, TRAP-induced platelet aggregation was unaffected. Summarily, these findings indicate that a fully automated coagulation analyzer can accurately and simultaneously quantify thrombin-induced platelet aggregation. The antiplatelet effect of dabigatran in NVAF patients could be detected by this method and provide novel treatment strategies for prevention of stroke.

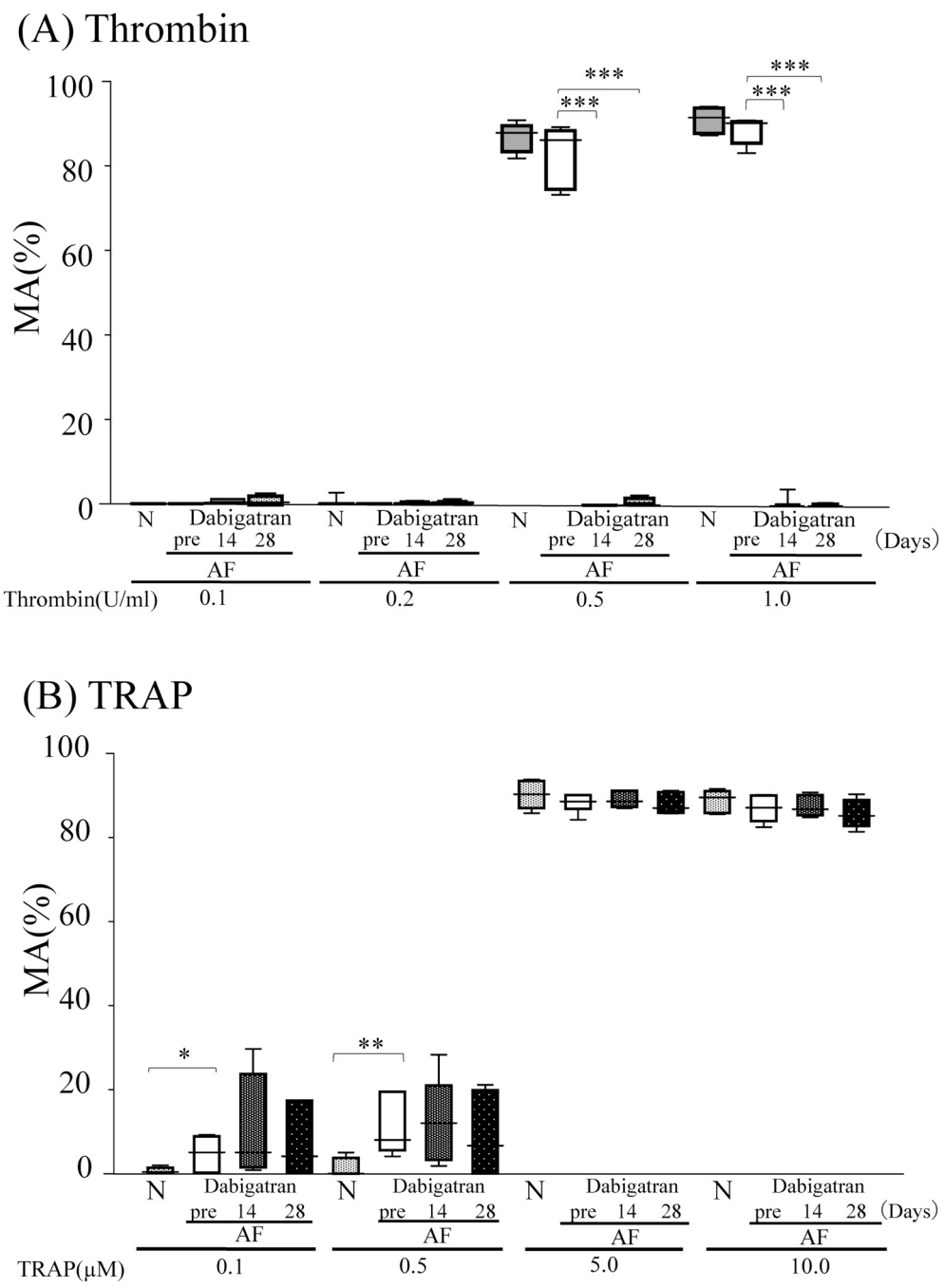

Fig. 1. Thrombin- and thrombin receptor activating peptide (TRAP)-induced platelet aggregation using automated platelet aggregometry during treatment with dabigatran in patients with non-valvular atrial fibrillation (NVAF). Gray bars indicate normal controls; white bars, before treatment with dabigatran in patients with NVAF; dark gray bars, 14 days after treatment; dot pattern bars, 28 days after treatment. (A) Thrombin-induced platelet aggregation was examined in the range of 0.1–1.0 U/mL thrombin. In a normal control group and before treatment with dabigatran, the median maximum platelet aggregation (MA %) with range (25th–75th percentile) induced by thrombin was elevated in a concentration-dependent fashion with thrombin. Platelet aggregation was completely inhibited by dabigatran treatment on days 14 and 28. (B) The results of TRAP-induced platelet aggregation (0.1–10.0 μM) were expressed in the same manner as thrombin-induced platelet aggregation. MA % was elevated in proportion to the concentration of TRAP, regardless of dabigatran treatment. TRAP-induced platelet aggregation did not differ among pretreatment and post-treatment measures at any concentration of TRAP. *** $p < .001$, ** $p < .01$, * $p < .05$.

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Declaration of competing interest

None.

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