Relation Between Dabigatran Concentration, as Assessed Using the Direct Thrombin Inhibitor Assay, and Activated Clotting Time/Activated Partial Thromboplastin Time in Patients With Atrial Fibrillation

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Dabigatran is a direct thrombin inhibitor that has been approved for preventing stroke in patients with atrial fibrillation. In this study, we aimed to assess the associations between the dabigatran concentration (calculated through plasma-diluted thrombin time, as assessed using the Hemoclot assay) and the activated partial thromboplastin time (aPTT) and activated clotting time (ACT). We recruited 137 patients with atrial fibrillation who were receiving a normal dose of dabigatran (300 mg/d) or a reduced dose of dabigatran (220 mg/d, usually administered to patients who were elderly, had moderate renal dysfunction, or who were also receiving verapamil). We then assessed the aPTT, ACT, and Hemoclot results of the patients and calculated the plasma dabigatran concentration. The mean plasma concentration of dabigatran was 127 ± 88 ng/ml, although no significant differences in dabigatran concentration, ACT, or aPTT were observed when we compared the 2 doses of dabigatran (300 or 220 mg/d). The dabigatran concentration was within the therapeutic levels in most patients, although a high value (>300 ng/ml) was observed in several patients, which indicated a high risk of bleeding. The dabigatran concentration was strongly and positively correlated with ACT and aPTT ($r = 0.87$, $p < 0.001$; and $r = 0.76$, $p < 0.001$; respectively). Multivariate analysis revealed that verapamil use was independently associated with elevated dabigatran concentrations ($p < 0.001$). Therefore, ACT and aPTT may be useful for bedside assessment of the anticoagulant activity of dabigatran, and verapamil use may be a risk factor for elevated dabigatran concentrations. **(Am J Cardiol 2015;115:1696−1699)**

Dabigatran is a direct thrombin inhibitor (DTI) that was recently approved for preventing stroke in patients with atrial fibrillation (AF). This approval provides new options for periprocedural anticoagulation in AF ablation[1,2] and direct current cardioversion for AF.[3] In addition, dabigatran does not require routine monitoring in clinical practice, given its predictable pharmacokinetic and pharmacodynamic profile.[4] However, the plasma dabigatran concentration cannot be directly measured, and no coagulation parameters reliably predict overexposure to dabigatran. Interestingly, a plasma-diluted thrombin time assay (DTI assay, the Hemoclot assay) has recently been used to measure the plasma concentration of DTIs.[5,6] Although DTI assays are not used to monitor dabigatran in clinical practice, activated partial thromboplastin time (aPTT) is a common method for measuring dabigatran's anticoagulation activity. As aPTT is a biomarker for intrinsic pathway activity,[7] the RE-LY (Randomized Evaluation of Long-term Anticoagulant Therapy) study reported that a trough aPTT of >80 seconds was associated with an increased risk of bleeding.[8] Similarly, activated clotting time (ACT) is used to monitor intrinsic pathway activity in whole blood and may also be a marker for the activity of dabigatran. Although van Ryn et al[5] have reported that dabigatran concentration is associated with ACT, no clinical study has confirmed this association. Therefore, we investigated the associations between dabigatran concentrations and aPTT/ACT and evaluated whether any patient characteristics were associated with dabigatran concentrations.

Methods

We enrolled 137 outpatients (65 ± 12 years old, 97 men) with AF who began receiving dabigatran after April 3, 2012, at the Cardiovascular Center, Yokosuka Kyosai Hospital. The normal dose was 300 mg/d, although a reduced dose of dabigatran (220 mg/d) was provided to patients who were elderly (≥70 years old), had moderate renal dysfunction (creatinine clearance 30 to 50 ml/min), or were also receiving verapamil. Patients with a low body weight also received the reduced dose at the attending physician's discretion. The Ethical Committee at Yokosuka Kyosai

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See page 1699 for disclosure information.

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Table 1
The characteristics of patients with atrial fibrillation who were treated with dabigatran

Variables	All cases (n = 137)	300 mg/day dabigatran (n = 41)	220 mg/day dabigatran (n = 96)	*P*-value
Age (years)	65.0 ± 11.7	60.3 ± 11.8	67.0 ± 11.2	0.002*
Men	71%	88%	64%	0.004**
Creatinine clearance (mL/min)	79.5 ± 26.3	91.8 ± 27.6	74.2 ± 24.0	<0.001*
Body weight (kg)	64.9 ± 10.8	69.5 ± 9.5	63.0 ± 10.8	0.001*

Data are expressed as mean ± standard deviation or as percentage.
P-values are calculated using an *unpaired *t*-test or **Fisher's exact test.

Hospital reviewed and approved this study's design, and all patients provided their written informed consent.

All patients in this study received their dabigatran at 6 A.M. and were followed up at either a morning (9 A.M.) or afternoon (12 noon) session for simultaneous evaluation of their DTI, aPTT, ACT, and serum creatinine levels (through a single blood sample). Therefore, the patients' blood samples were obtained once, at 3 or 6 hours, after the oral administration of dabigatran, on an outpatient basis. The patients' aPTT (normal range 25 to 35 seconds) values were determined using a ThromboCheck aPTT assay (Sysmex Corp., Hyogo, Japan), and their ACT values were determined using a Hemochron assay (International Technidyne Corp., Edison, New Jersey).

The Hemoclot thrombin inhibitor assay (Hyphen-BioMed, Neuville-Sur-Oise, France) is a sensitive diluted thrombin time assay that allows for the quantitative measurement of DTI activity in plasma, based on the inhibition of a defined concentration of thrombin. This test was used to evaluate dabigatran plasma concentrations by diluting the test plasma (1:8) with normal, pooled human plasma. Clotting was initiated by adding a constant amount of highly purified human α-thrombin. We also used Dabigatran Plasma Calibrators (Aniara, Mason, Ohio), which consist of pooled, normal plasma with known amounts of dabigatran, to establish the dabigatran calibration curve. The patients' dabigatran concentrations were then calculated by comparing their diluted thrombin time to the calibration curve.

All data were expressed as mean ± SD or frequency, and the correlations between the dabigatran concentrations and coagulation markers (ACT and aPTT) were evaluated using the Pearson's correlation coefficient (r). We then stratified these parameters according to the patients' characteristics, including age, gender, creatinine clearance, and body weight and compared the various groups through the unpaired *t* test. Multiple regression analysis was performed to identify the independent factors that influenced dabigatran concentrations after adjusting for age, gender, body weight, creatinine clearance, and the use of verapamil. All statistical analyses were performed using SPSS software (version 18; SPSS Inc., Chicago, Illinois); all tests were 2 tailed, and p values <0.05 were considered statistically significant.

Results

Of the 137 subjects, 96 received a reduced dose of dabigatran (Table 1). Significant differences in age, gender, renal function, and body weight were observed when we compared the patients who received the low and normal doses of dabigatran. The mean plasma concentration of dabigatran was 127 ± 88 ng/ml (range 10 to 430 ng/ml; Figure 1, Table 2), and the mean ACT and aPTT values were 188.4 ± 31.5 and 45.0 ± 7.8 seconds, respectively. No significant differences were observed in these parameters when we compared the 2 doses of dabigatran (300 vs 220 mg/d) or the 2 sampling times (3 vs 6 hours). The dabigatran concentrations were significantly and positively correlated with ACT (r = 0.87, p <0.001) and aPTT (r = 0.76, p <0.001). The dabigatran concentrations were not correlated with age or creatinine clearance, although a moderate negative association was observed between the dabigatran concentrations and body weight (r = −0.176, p = 0.039; Table 3). Of the patient characteristics that were included in the multivariate analysis, only the use of verapamil was significantly and independently associated with the dabigatran concentrations (p <0.001; Table 4).

Discussion

The DTI assay is a sensitive assay that allows for the quantitative measurement of DTI activity in plasma, based on the inhibition of a fixed concentration of thrombin. As a result, there is a linear correlation between the dabigatran concentration and the clotting time that is measured using this assay. Although the relation between clotting time and dabigatran has been described previously,[5] to our knowledge, this is the first study to quantify dabigatran concentrations through the Hemoclot assay. In addition, the distribution of DTI assay values in patients who are receiving dabigatran for AF has not been reported. Therefore, the present study is the first to report the dabigatran concentrations, as measured using a DTI assay, in patients with AF who are receiving outpatient treatment.

In the Japanese subgroup of the RE-LY study, the median peak steady-state dabigatran concentrations in patients with AF were approximately 150 ng/ml (high dose: 150 mg twice daily) and 95 ng/ml (low dose: 110 mg twice daily).[9] Similarly, we found that the dabigatran concentrations in most of our patients were within this therapeutic range, although several patients had concentrations of >300 ng/ml, which may indicate an increased risk of bleeding. One possible explanation for this variability may be the low bioavailability (6.5%) of dabigatran,[4] which would suggest that minor changes in its absorption would significantly affect its plasma concentration. The low bioavailability of dabigatran may also explain the absence of any significant difference in the plasma concentrations of dabigatran at the 2 doses at the various blood draw time points. In addition, a reduced dose of dabigatran (220 mg/d) was provided to patients who were elderly, had moderate renal dysfunction, or were also receiving verapamil in this study. As a result, the concentration of dabigatran in patients with a reducing dose was relatively high and did not differ from that of patients with the usual dose.

We also observed a strong and positive correlation between dabigatran concentrations and ACT. The qualitative assay for ACT evaluates intrinsic pathway activity, based on a testing principle that is similar to that for aPTT, although the