Effect of dabigatran on the volume of intracerebral hemorrhage in ischemic and hemorrhagic stroke of rats

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It seems like just yesterday that I started my master's degree, and the finish line is around corner. Lock back my life of master's degree, I was very naive and young. However, I become more mature academically, because I endured that all time.

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Now, I have graduation ahead and also am standing in front of new starting line. Experience of master’s degree would become stepping stones for being a modest scientist who keep my first intension.

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ABSTRACT

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Anticoagulants are effective for several thrombotic diseases including atrial fibrillation and venous thrombosis. Warfarin has been widely used. However, the use of warfarin increases the risk of hematoma expansion as well as the development of intracerebral hemorrhage (ICH). Hemorrhagic transformation nay also occur after ischemic stroke, which is associated with the loss of vascular integrity. Dabigatran, a direct thrombin antagonist, has shown to be superior to warfarin in reducing stroke in patients with nonvalvular atrial fibrillation. Particularly, the risk of ICH is remarkably reduced in the dabigatran users. However, it is uncertain whether the volume of hematoma is different between warfarin users and dabigatran users when ICH develops in conditions with the loss of vascular integrity. only little evidence provided about relation of dabigatran and ICH expansion. This study aimed at comparing the occurrence and expansion of intracerebral hematoma in rats pretreated with warfarin or dabigatran by inducing focal ischemia using a middle cerebral artery occlusion (MCAO) and by inducing ICH using

intracerebral injection of collagenase. Eight-week-old male Wistar rats were randomly received 0.2 mg/kg of warfarin, 20 mg/kg of dabigatran, or normal saline. After inducing MCAO or collagenase-induced ICH, the volume of ICH was assessed using spectrometric hemoglobin assay. The vascular integrity was examined by measuring the density of basement membrane on transmission electron microscopy. After MCAO, ICH was observed more frequently in the warfarin group (12 of 18, 66.7%) than the dabigatran group (6 of 27, 22.2%, $p=0.003$) and the placebo (5 of 36, 12.9%, $p<0.0001$). However, the amount of hemoglobin was not different between the groups. The density of basement membrane determined on the infarct core and penumbra area was not different between the groups. In the collagenase-induced ICH model, the amount of hemoglobin was similar between the groups. In conclusion, ICH less frequently developed in dabigatran-pretreated rats than in warfarin-pretreated rats when large cerebral infarction was induced. However, the volume of ICH was similar in rats with ICH. The degradation of basement membrane may not be the main cause of different risks of ICH between warfarin users and dabigatran users.

Key words : Stroke, hemorrhage, warfarin, dabigatran, hematoma, basement membrane

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I. INTRODUCTION

Anticoagulants are effective for preventing ischemic stroke and systemic embolism in patients with several thrombotic diseases including atrial fibrillation and venous thrombosis. However, use of anticoagulants increases the risk of bleeding including intracerebral hemorrhage (ICH).[1] This anticoagulant-associated ICH may be fatal in about 50% of patients.[2] Hemorrhagic transformation may also occur after ischemic stroke, which is associated with loss of vascular integrity. Anticoagulation may increase the risk of ICH. In fact, anticoagulation is not recommended during hyperacute stage of large cerebral infarction.

Warfarin is a vitamin-K antagonist, which inhibits coagulation factor II, VII, IX, and X. Although warfarin has been widely used, the use of warfarin increases the risk of hematoma expansion as well as the development of ICH.[3] Recently direct anticoagulants (DOAC) that inhibit thrombin (diabigatran) or factor Xa (rivaroxaban, apixabian, and

edoxaban) have shown the superiority over warfarin in reducing stroke or systemic embolism. A metaanalysis demonstrated a 19% relative risk reduction of stroke or systemic embolic events.[4] The risk of ICH was also remarkably reduced by 51% in the DOAC users comparing with warfarin users.

Spontaneous ICH may occur by arterial rupture. However, the disruption of vascular integrin may induce hemorrhagic transformation and ICH. Ischemic injury of the brain leads to the loss of vascular integrity and may cause hemorrhagic transformation. Thrombin induces a blood-brain barrier disruption and the loss of vascular integrity,[5] which may contribute to the development of ICH. Warfarin and DOAC may be responsible for the hematoma expansion in arterial rupture-associated ICH by inhibiting normal hemostasis. In contrast, DOAC may play role in protecting from the loss of vascular integrity by inhibition thrombin.[6]

Although the risk of ICH is lower in dabigatran users than in warfarin users, it is uncertain whether the volume of hematoma is different between them when ICH develops in conditions with the loss of vascular integrity. Intracerebral injection of bacterial collagenases results in ICH by degrading collagen type IV, which is a component of vascular basement membrane. Therefore, we investigated the occurrence and expansion of intracerebral hematoma after taking warfarin and dabigatran in two different animal models that may induce ICH by vascular integrity disruption, middle cerebral artery occlusion (MCAO) for ischemic stroke and collagenase induced ICH model for hemorrhagic stroke.

II. MATERIALS AND METHODS

1. Experiment animals

All experiments were performed under the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The protocols were approved by the Yonsei University College of Medicine Institutional Animal Care and Use Committees (IACUC, protocol N-2015-0186). Eight-week-old male Wistar rats weighing 280-310g were used for all experiments. Animals were housed in a temperature-controlled animal facility under a 12/12 hr reversed light and dark cycle, and could freely access to food and water. Animals were randomly numbering for double blinding experiment in order not to reflect subjective view of experimenter.

2. Study group and drug administration

Rats were randomly received warfarin (0.2 mg/kg/day, Daewha Pharmaceutical Co., Gangwon, Korea), dabigatran (20 mg/kg/day, Boehringer Ingelheim, Ingelheim, Germany), or normal saline. The doses of warfarin and dabigatran were determined based on those of previous study[7]. Warfarin was administered orally using a zonde once a day and dabigatran twice a day for 7 days. In this drug treatment, we gave dabigatran oral administration two times. So, saline group and warfarin group were taken saline once more after drug administration for the same conditions. The last drug oral administration was 30 min before the operation. For randomizing all drugs, 0.0025% of starch from wheat (S5127, sigma-Aldrich corp., St. Louis, MO) was added in saline and both drugs to make solutions unclear. One researcher prepared drug with 0.0025% of starch and gave random codes to each drugs. In this blinded situation, experimenter

treated randomized drug to animals until experiment ended.

3. INR measurements

To measure international normalized ratio (INR), blood was collected from the tale vein into a syringe containing 0.1 ml citrate. Blood was drawn after 30 min of last drug administration. Plasma was obtained by centrifugation at 700 g for 15 min at 4℃. INR was measured at Green cross Lab cell (Gyounggi, Korea).

4. Quantitative method for the volume of intracerebral hemorrhage

General anesthesia was induced by inhalation of 4% isoflurane delivered in 70% nitrous oxide and 30% oxygen and maintained with 2% isoflurane with same gas mixture. Body temperature was monitored through a rectal probe and maintained at 37.0 ± 0.5℃ continuously during the surgery using a homeothermic blanket control unit and a heating pad (Harvard Apparatus, Holliston, MA). Animals were placed on a stereotaxic (Benchmark Leica biosystems, Wetzlar, Germany) head-holder. Midline skin incision was made to reveal the bregma. A 1-mm burr hole was drilled at the left cranial vault 3 mm lateral from the bregma. A 5 ul syringe with a 26-gauge needle (Hamilton Company, Reno, NV) was inserted into the caudate-putamen (6.5 mm below the surface of the skull) through the burr hole. 1, 2, 4, 8, 10 ul of blood were injected to show injection method delivering solutions without problems. The needle was left in place for 10 min after the injection of BC to prevent reflux of BC solution during the withdrawal of a needle. The skin incision was closed with 3M Vetbond tissue adhesive for wound (1469SB, 3M Health Care, St.Paul, MN).

5. Experiment 1 - Middle cerebral artery occlusion

General anesthesia was induced by inhalation of 4% isoflurane delivered in 70% nitrous oxide and 30% oxygen and maintained with 2% isoflurane with same gas mixture. Body temperature was monitored through a rectal probe and maintained at 37.0 ± 0.5℃ continuously during the surgery using a homeothermic blanket control unit and a heating pad. Under the operating microscope (Carl Zeiss, Inc., Thornwood, NY, USA), midline incision was made, vagus nerve was separated with the left common carotid artery(CCA) which located beside of trachea carefully. CCA was ligated first, the external carotid artery (ECA) was ligated continually. , The occipital artery(OCCA) and Pterygopalatine artery(PPA) which were splited from proximal of the internal carotid artery (ICA) were ligated together with 6-0 silkam silk suture (B.Braun Medical, Rubi, Spain) to block blood flow. OCCA was cauterized. ICA was clipped with microclamp temporarily and branch of CCA was punctured with microscissor to make a small hole. Though the hole, the middle cerebral artery (MCA) was occluded by silicon rubber-coated nylon thread (Doccol corporation, Sharon, MA) at the origin of the left MCA bifurcation. Rats were sacrificed after 22 hr reperfusion following two hour MCA occlusion.[8] [9,10] Collected brains were sectioned 1mm thickness and arranged in regular sequences on clear acryl plate with ice cooling. Two experimenters who did not know about blind code determined intracerebral hemorrhage and subarachnoid hemorrhage separately.

6. Experiment 2 - Induction of intracranial hemorrhage by collagenase injection

General anesthesia was induced by inhalation of 4% isoflurane delivered in 70% nitrous oxide and 30% oxygen and maintained with 2% isoflurane with same gas mixture. Body temperature was monitored through a rectal probe and maintained at 37.0 ± 0.5℃

continuously during the surgery using a homeothermic blanket control unit and a heating pad (Harvard Apparatus, Holliston, MA). Animals were placed on a stereotaxic (Benchmark Leica biosystems, Wetzlar, Germany) head-holder. Midline skin incision was made to reveal the bregma. A 1-mm burr hole was drilled at the left cranial vault 3 mm lateral from the bregma. A 5 ul syringe with a 26-gauge needle (Hamilton Company, Reno, NV) was inserted into the caudate-putamen (6.5 mm below the surface of the skull) through the burr hole. A total 1 μl (0.025U) of bacterial collagenase (BC) Type IV (C1889, Sigma-Aldrich co., St. Louis, MO) was injected for 5 min with a micro-infusion pump (Kdscientific, Holliston, MA). The needle was left in place for 10 min after the injection of BC to prevent reflux of BC solution during the withdrawal of a needle. The skin incision was closed with 3M Vetbond tissue adhesive for wound (1469SB, 3M Health Care, St.Paul, MN). Collected brains were sectioned 1mm thickness and arranged in regular sequences on clear acryl plate with ice cooling. Two experimenters who did not know about blind code determined intracerebral hemorrhage and subarachnoid hemorrhage separately.

7. Transmission electron microscopy

The ultrastructure of basement membrane was examined using transmission electron microscopy (TEM). Animals were sacrificed by cardiac perfusion. The third and fifth blocks were used for 2% 2,3,5-triphenyltetrazolium chloride staining to determine infarction and penumbra areas. The fourth blocks of the brain were pre-fixed with glutaraldehyde in 2% paraformaldehyde, washed in 0.1M phosphate buffer (pH 7.4). and then post-fixed in 1% osmium tetroxide in the same buffer for 15 minutes.[11] After dehydration through a graded series of ethanol and exchange with propylene oxide,

tissues were embedded in a mixture of Epon and cut into pieces of 1 mm^3. Uultrathin sections were obtained by ultramicrotome (Ultracut UCT; Leica, Australia) with a diamond knife. The density of the basement membrane was measured using Scionimage (Scion corporation, Maryland, USA) in the infarct core of basal ganglia and the penumbra region of ipsilateral hemisphere.

8. Spectrophotometric hemoglobin assay

The amount of ICH was measured using a spectrophotometric hemoglobin assay. Twenty four hours after the induction of ICH, rats were sacrificed by cardiac perfusion and decapitated. The brains were separated into left and right hemispheres. Olfactory bulb and cerebellum were removed. Each hemisphere was homogenized with 1 ml of red blood cell lysis buffer (Qiagen, Maryland, USA) and 2 ml of PBS until whole tissues were minced. After homogenization, ultrasound was applied for 3 min to lyse erythrocytic membranes. After centrifugation at 20000 rpm for 30 min at 4℃, 250 μl of supernatant was mixed with 1000 ul of Drabkin's reagent (D5941, Sigma-Aldrich, St.Louis, MO, USA) and reacted for 15 min. Absorption rate was determined at 540 nm using a spectrophotometer.

9. Neurologic deficits

Neurologic evaluation was performed before and after MCAO and before sacrifice. All surviving animals were graded based on Garcia's scale and Longa's scale (Garcia et al., 1995, Longa et al., 1989). Briefly, six items were evaluated for Garcia test. Zero to three points were given to each item when an animal had a deficit and summed for a total score. The minimum neurologic score was 3 and the maximum was 18. The items

evaluated were 1) ability to approach all four walls of the cage, 2) symmetry in the movement of four limbs, 3) forepaw outstretching, 4) climbing and gripping abilities on the wire cage, 5) reaction to stimulus on both sides of body, and 6) response to vibrissae touch.[12] For Longa's scale, four items were evaluated. Minimum neurologic score was 0 and the maximum was 4. The items evaluated were 1) Holding edge of table, 2) failure to extend the left forepaw fully, 3) circling to the left, and 4) no spontaneous walking with a depressed level of consciousness.[13]

10. Statistical analysis

Statistical analyses were performed using SPSS (version 23.0, SPSS Inc., Chicago, IL, USA). The normality of distributions was verified using the Kolmogorov-Smirnov test. Differences between the groups were compared with a one-way analysis of variance (ANOVA) test, followed by a post-hoc Tukey method (hematoma volumes, PT and INR values). The differences between the groups were compared by a Kruskal-Wallis test (Neurologic deficit scores). The Chi-square test or Fisher's exact test was used to compare categorical variables (mortality rates, incidence rates of HT) between the groups. Values were presented as a mean ± standard deviation (SD). $P < 0.05$ was considered significant.

III. RESULTS

In this study, total 168 rats were used. Thirty animals were used for the validation of spectrophotometric hemoglobin assay in induced ICH, 81 animals were used for the MCAO model, and 45 rats were used for the ICH model.

1. Validation of the quantitative method for the volume of intracerebral hemorrhage

We determined Hb levels after the stereotaxic administration of various amounts of blood into the brain. There were strongly positive correlation between the Hb concentration and blood volume($r=0.884$, $p<0.001$). Injection of 1 ul (0.0482 ± 0.0017 g/dl, $p = 0.996$) or 2 ul blood (0.0622 ± 0.0047 g/dl, $p = 0.110$) into the brain did not cause any significant increases in the Hb level compared to the normal saline control (0.0454 ± 0.0042 g/dl). The Hb level was significantly increased at 4 ul (0.0704 ± 0.0026 g/dl, $p = 0.004$) and increased in an injection volume-dependent manner. The Hb levels were 0.082 ± 0.01 g/dl at 8 ul ($p < 0.001$) and 0.098 ± 0.018 g/dl at 10 ul ($p < 0.001$).

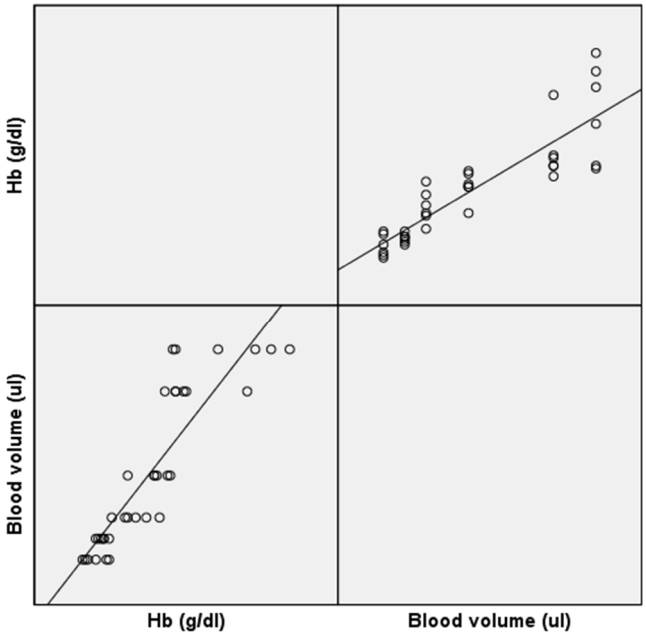

Figure 1. Mean blood volume of blood injection in normal animals in 5 different blood volumes.

2. Experiment 1 - Middle Cerebral Artery Occlusion

A. Incidence rate of intracerebral hemorrhage after the induction of middle cerebral artery occlusion

Twenty four hours after the induction of MCAO, ICH was observed in 13.9% (5 of 36 animals) of the placebo group, 22.3% (6 of 27 animals) of the dabigatran group, and 66.7% (12 of 18 animals) of the warfarin group. The rate of ICH was significantly higher in the warfarin group than the placebo group ($p<0.0001$) or the dabigatran group ($p=0.003$). Warfarin group showed 3-fold higher rate of ICH than the dabigatran group. However, there was no difference of the ICH rate between the placebo and the dabigatran group ($p=0.389$) (Fig. 2).

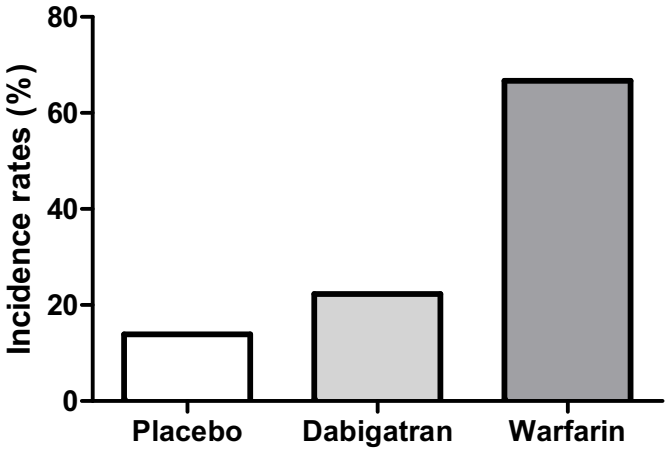

Figure 2. Incidence rates of intracerebral hemorrhage after the induction of middle cerebral artery occlusion.

B. Hematoma volume

The amount of hemoglobin in the brain was determined from animals that showed ICH. The amount of hemoglobin was not different between the groups (0.072 ± 0.0014 g/dl in the placebo group, 0.129 ± 0.077 g/dl in the dabigatran group, and 0.074 ± 0.026 g/dl in the warfarin group, (P = 0.183).

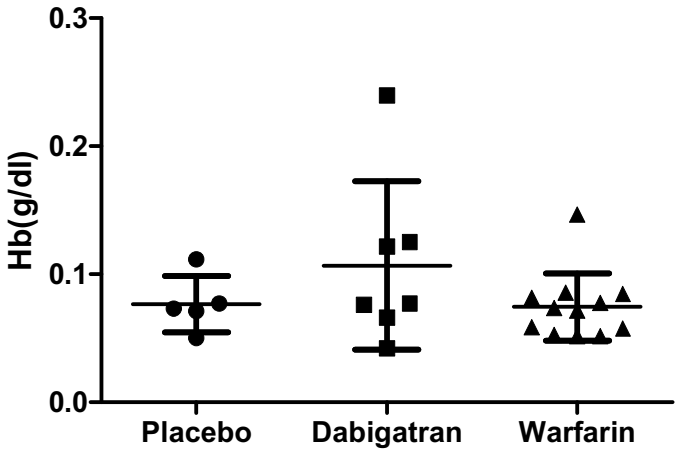

Figure 3. Amount of hemoglobin in the brain with visible intracerebral hemorrhage.

C. Basement membrane density

The density of basement membrane was determined in the infarct core (basal ganglia) and penumbra areas of ipsilateral hemisphere. There was no difference in the density among the groups.

Table 1. The density of basement membranes in each group.

V		Placebo	Dabigatran	Warfarin	P-value
Vat	Infarct-core	115.0 ± 24.5	119.2 ± 28.7	107.0 ± 29.6	0.150
	Penumbra	140.8 ± 27.8	141.8 ± 31.2	134.9 ± 36.6	0.671

Values are mean ± standard deviation.

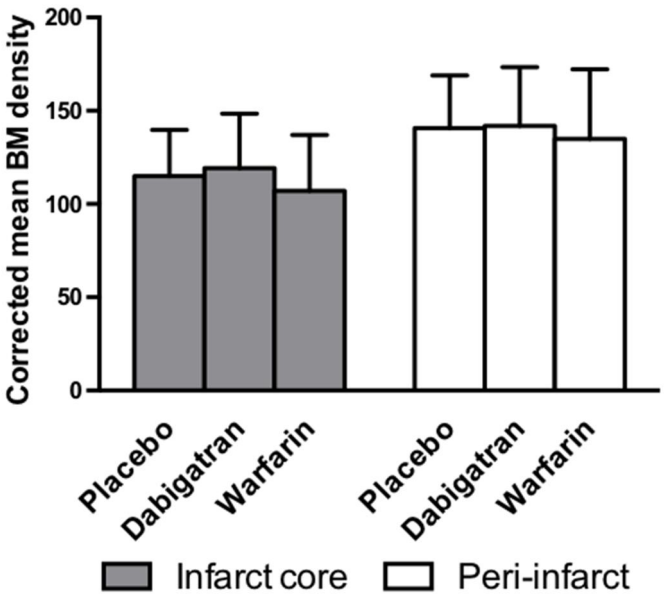

Figure 4. The density of basement membrane measured in the infarct-core and penumbra.

D. INR kinetics

Blood samples were available in 33 of the placebo, 26 of the dabigatran, and 16 of the warfarin group. INR values were significantly higher in the warfarin group (6.3 ± 1.9) than the dabigatran group (1.8 ± 0.4, p<0.0001) or the placebo group (1.6 ± 0.5, p<=0.0001). INR levels did not differ between the placebo group and the dabigatran group (p=0.857). (Fig. 4)

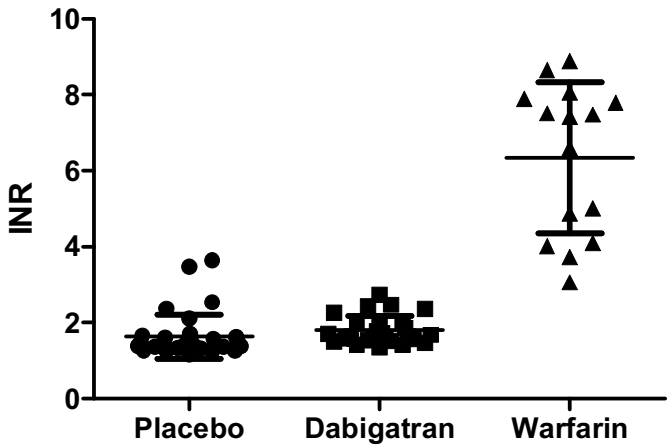

Figure 5. INR values in blood samples obtained 1 hr after the last oral administration of study drugs.

E. Neurologic outcome and mortality

Mortality rate at 24 hrs after MCAO did not differ between placebo group and dabigatran group (p=0.693). Mortality rate was 6 % (1 of 18) in the placebo group, 12 % (2 of 18) in the dabigatran group, and 94 % (17 of 18) in the warfarin group. Neurologic deficits were assessed in survived rats (28 in the placebo, 23 in the dabigatran, and 1 in the warfarin group). Longa's scale is a 5-point grading system (0 to 4), and higher scores mean more severe neurologic deficits. Garcia's scale grades from 0 to 18 points, and lower scores mean more severe neurologic deficits. There was significant difference between placebo group and dabigatran group on Garcia's scale (13.6 ± 3.8 vs. 11.3 ± 3.7, p=0.017, Fig. 6). The degree of neurologic deficits was not different between the placebo group and the dabigatran group neither on Longa's scale (1.6 ± 0.9 vs. 2.1 ± 0.9, p=0.152, Fig.7).

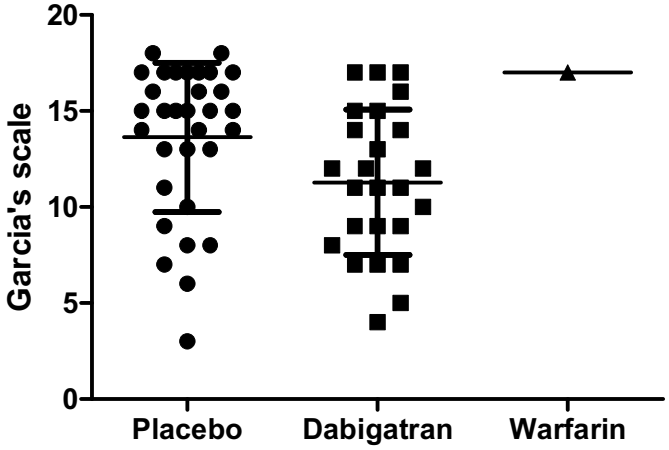

Figure 6. Neurologic deficits assessed by Garcia's test. Minimum score is 0 and maximum score is 18.

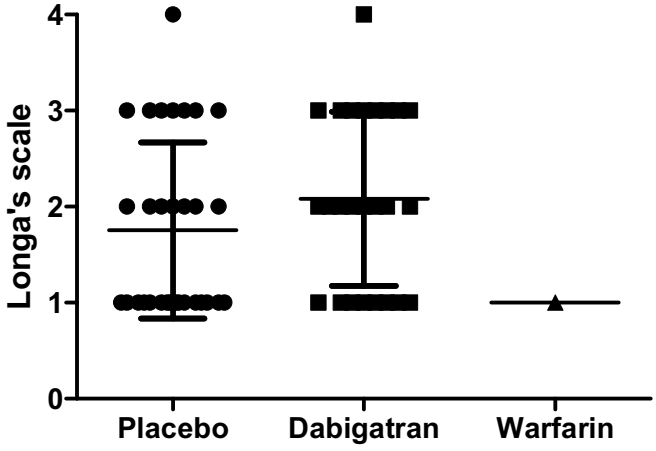

Figure 7. Neurologic deficit tested by Longa's scale. Minimum score is 0 and maximum score is 4.

3. Experiment 2 - Collagenase induced ICH

A. Collagenase induced intracerebral hematoma volume

Twenty-four hours after hemorrhage induction by collagenase type IV, Compared to the placebo group (0.14 ± 0.04g/dl), distinct size difference of hematoma formation in intracerebral hemorrhage in warfarin (0.16 ± 0.03g/dl) and dabigatran (0.16 ± 0.05g/dl) group was not observed, (P= 0.364, n=14 per group).

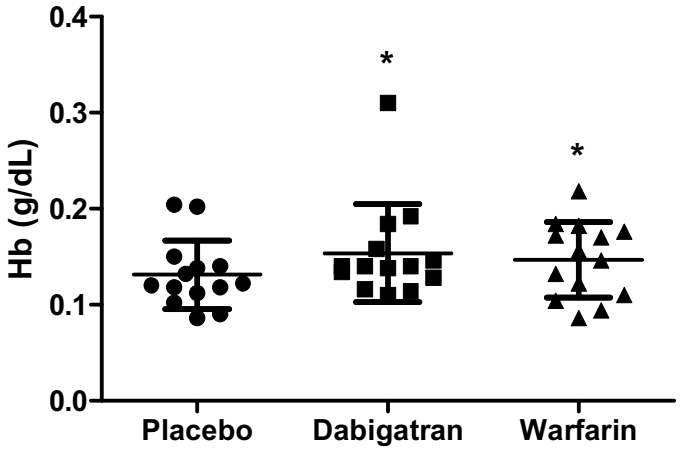

Figure 8. Hemorrhagic blood volume measured 24hour after hemorrhage induction by quantitative hemoglobin content determination in placebo, dabigatran, warfarin group. P>0.05 compared to placebo group; error bars indicate SD.

B. Basement membrane density

Basement membrane density did not differ significant among all three groups (p=0.116). However, basement membrane density of warfarin group (137.2 ± 23.9) is slightly lower than placebo (122.8 ± 37.0, p=0.631) or dabigatran group (116.4 ± 38.2, p=0.148).

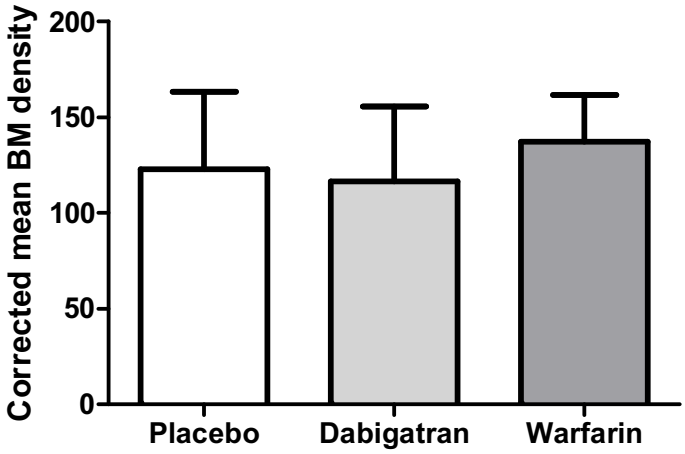

Figure 9. Basement membrane (BM) density measured from peri-hematoma area of ipsilateral hemisphere.

C. INR Kinetics

Blood samples were collected in 14 of each the placebo, dabigatran and warfarin group. There were significant differences between the groups ($P<0.0001$) in INR. Mean INR values were found to be 1.5 ± 0.3 (n=14) in placebo group and after 24 hrs of dabigatran administration, INR values were 2.1 ± 0.4. Between placebo and dabigatran group had significant differences in INR ($P=0.048$). After 24 hrs of warfarin administration, INR values were elevated (3.5 ± 1.1, n=14). Some warfarin treated animals' INR values were higher than human's therapeutic range. In contrast, INR values of dabigatran group were almost in the similar range of human's therapeutic range compare with warfarin. (Fig. 10)

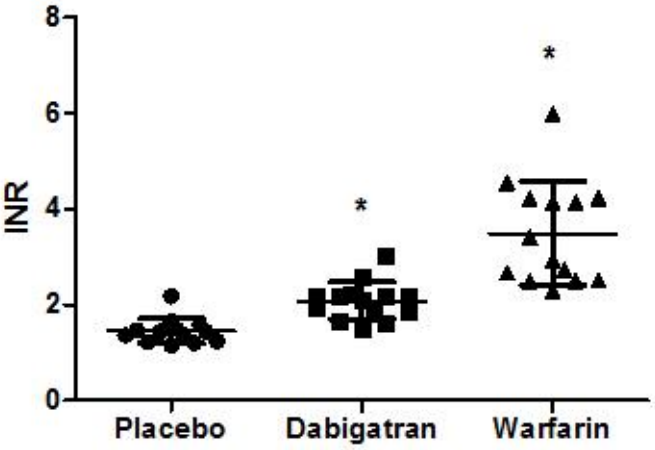

Figure 10. INR values were determined 1hr after last oral administration of dabigatran and warfarin treatment.

D. Mortality

Mortality rate at 24 hr after ICH induction was 7% (1 of 14) in placebo, 21.4% (3 of 14) in dabigatran group, 50% (7 of 14) in warfarin group. Warfarin group showed higher mortality rate than other two groups, more than 2.4 folds than dabigatran group, cause of death was considered by serious bleeding from surgical part. There were no significant difference in all groups (P=0.055).

IV. DISCUSSION

The risk of intracerebral hemorrhage is remarkably lower in dabigatran users than in warfarin users. This study investigated a hypothesis that the volume of intracranial hemorrhage is similar between warfarin-pretreated rats and dabigatran-pretreated rats, although the risk of ICH is lower in dabigatran-pretreated rats.

We first examined the frequency of ICH after the induction of cerebral infarction. Hemorrhagic transformation often occurs in patients with large cerebral infarction. The risk of ICH increases in warfarin users when large cerebral infarction develops.[14] In the thromboembolic model of mice, dabigatran did not increase secondary hemorrhage after IV thrombolysis.[15,16] However, little information is available whether the risk of ICH is lower in dabigatran users comparing with warfarin users when cerebral infarction develops. This study showed that the frequency of ICH after the induction of large cerebral infarction was significantly lower in the dabigatran-pretreated rats than the warfarin-pretreated rats. In addition, the frequency of ICH was not different between dabigatran-pretreated rats and placebo in this study. A previous study in mice with transient MCAO, dabigatran pretreatment did not increase the risk of hemorrhagic transformation when comparing with control mice,[17] which was consistent with findings in this study. Previously, the risk of ICH was lower in dabigatran users than in warfarin users among non-stroke patients. Our findings provide an additional evidence that the risk of ICH may be lower in dabigatran users than warfarin users when ischemic stroke develops. Current guidelines do not recommend to use anticoagulants in very early stage of ischemic stroke due to the risk of ICH.[18] However, these recommendations were based on evidence from older generation of anticoagulants. It remains uncertain whether direct oral anticoagulants can be safely used during very early stage of

infarction. Our findings also suggest that dabigatran may be used safely in these groups of patients.

We then assessed the amount of hemoglobin in the brain of rats with visible ICH after the induction of cerebral infarction. The amounts of hemoglobin were similar between dabigatran-pretreated rats and warfarin-pretreated rats. We further compared the volume of ICH between dabigatran-pretreated rats and warfarin-pretreated rats using a well-known ICH model that is a collagenase-induced ICH model. Collagenase type IV is one of structural proteins for basement membrane that act for a mechanical barrier.[19] In the collagenase-induced ICH model, hemorrhage develops by the collagenase-induced degradation of vascular basal lamina,[19] and resulting the loss of vascular integrity.[17,20] In this model, the amounts of hemoglobin were not different between dabigatran-pretreated rats and warfarin-pretreated rats. Findings in this study suggest that once the ICH develops, the amount of bleeding is not different between the dabigatran-pretreated rats and warfarin-pretreated rats, although the risk of ICH is lower in dabigatran-pretreated rats than in warfarin-pretreated rats.

Although it remains uncertain why the risks of ICH are lower in dabigatran users than warfarin users, dabigatran was suggested to act on neurovascular unit. Dissociation between astrocyte endfeet and the microvascular basal lamina or pericytes, which was observed in warfarin-pretreated rats after MCAO and subsequent tPA infusion, was improved in dabigatran-pretreated rats. Dabigatran completely blocked the effect of thrombin on increased endothelial cell permeability induced by thrombin treatment. Matrix metalloproteinase-9 (MMP-9) is a key proteinase that plays a role in hemorrhagic transformation after cerebral ischemia in the brain by degrading extracellular molecules of microvascular basal lamina.[7,21]

Warfarin pretreatment increased MMP-9 in the ischemic hemisphere comparing with placebo, however, there was no difference of MMP-9 activity between dabigatran-pretreated rats and placebo.[15,22] However, in this experiment, the basement membrane density in the ischemic core and penumbra area was not different between placebo, warfarin-pretreated rats and dabigatran-pretreated rats. This finding suggests that difference in the basement membrane degradation between dabigatran-pretreated rats and warfarin-pretreated rats might not be the main mechanism of reduced risk of ICH in dabigatran users. The degree of basement membrane degradation determined by basement membrane density correlates with the MMP-9 activity.[11] The mechanism of hematoma expansion after the development of ICH may be different from the cause of ICH. Hemostatic mechanisms, that inhibit coagulation, could be more important in the hematoma expansion. Given that anticoagulating effects are not much different between warfarin and dabigatran, the volume of ICH may not be much different when ICH develops.

In this study, the amount of hemoglobin was not different between the placebo, warfarin, and dabigatran groups in the collagen-induced ICH model. INR levels were remarkably higher in the warfarin-pretreated rats, which suggests that subtherapeutic anticoagulation was not the reason. In the previous studies, the volume of ICH that was assessed based on the spectrophotometric hemoglobin assay increased in warfarin-pretreated animals.[16] However, in a study based on sequential magnetic resonance imaging MRI taken 24 and 48 hr after the induction of collagenase-induced hemorrhage, the volume of hematoma was not different between the placebo, dabigatran, and warfarin groups[23], which was consistent with findings in this this study. The reason of this discrepancy is unknown. Further studies are necessary for this issue. In addition, the mechanism of ICH in the collagenase-induced model is different

from ICH in human stroke. While proteinase-induced basal lamina degradation of microvessels is the main cause of ICH in the former, the rupture of the artery or arteriole is the cause of the latter. Therefore, this different mechanisms between ICH in human and that in animals induced by collagenase should be considered for the interpretation of findings in experimental animals.

V. CONCLUSION

ICH less frequently developed in dabigatran-pretreated rats than in warfarin-pretreated rats when large cerebral infarction was induced. The frequency of ICH was not different between dabigatran-pretreated rats and placebo. However, the volume of hematoma was not different between rats pretreated with placebo, dabigatran, and warfarin, once ICH developed. The different mechanisms might play a role between the development and expansion of ICH in antiocoagulation-associated ICH.

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ABSTRACT (INKOREAN)

흰 쥐의 허혈성과 출혈성 뇌졸중에서 뇌내출혈 크기에 대한 다비가트란의 효과

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안 선 호

항응고제는 정맥 혈전증과 심방세동을 포함한 여러 혈전증을 앓는 환자에서 허혈성 뇌졸중과 조직 색전증을 예방하는데 효과적이다. 그러나 항응고제의 사용은 뇌내출혈을 포함한 출혈 발생의 위험성을 높인다. 항응고제와 관련된 뇌내출혈은 환자의 약 50%정도에서 치명적인 영향을 미친다[2]. 또한 뇌출혈은 허혈성 뇌졸중 이후에 발생할 수 있으며 혈관 안정성 손상을 부를 수 있다. 혈뇌장벽의 손상이 혈관성 뇌부종을 일으킬 수 있고, 미세혈관 내피세포 팽창과 별아교세포 종단의 급격한 분리와 팽창도 진행되기 때문이다. 항응고제를 투여받고 있는 환자에서 뇌출혈이 발생하면 초기 지혈이 안되어 출혈양이 더 많을 수 있다. 실제로 항응고제는 큰 뇌경색의 초급성 단계에서 주로 권장되지 않는다.

와파린은 응고인자 II, VII, IX와 X를 억제시키는 비타민 K의 길항제이다. 비록 와파린이 널리 사용되어 왔지만, 와파린의 사용은 혈종 확대의 위험성

뿐만 아니라 뇌내출혈 발생까지 높인다.[3] 최근 트롬빈을 억제하는 다비가트란이나 응고인자 Xa를 억제하는 리바록사반, 아픽사반, 에독사반과 같은 직접적 항응고제 (DOAC)가 뇌졸중이나 조직 색전증을 줄이는데 와파린보다 우세를 보이고 있다[4]. 이 중 다비가트란은 혈액응고단계에서 피브리노겐을 피브린으로 변환시키는 트롬빈을 직접적, 경쟁적으로 억제함으로써 혈전 생성을 막는다. 와파린을 처방 받은 환자와 비교하여 DOAC을 처방 받은 환자에서 뇌내출혈이 51% 정도 줄어들었다. 그러나 혈관 안정성 손실과 함께 뇌내출혈의 발생 시 와파린 섭취 환자와 다비가트란 섭취 환자 사이의 혈종 크기의 차이는 아직 확실하지 않다. 다비가트란과 뇌내출혈 확장의 연관관계는 아직 많이 밝혀지지 않고 있다. 이번 연구는 중대뇌폐색동맥(MCAO)과 콜라게나아제로 유도된 뇌내출혈(ICH) 동물모델을 통해 와파린과 다비가트란을 선처리한 흰 쥐에서 혈종의 확대와 발생에 대해 비교해보고자 한다.

8주령 수컷 Wistar 흰 쥐들에게 일반 생리식염수, 0.2mg/kg의 와파린과 20mg/kg의 다비가트란을 무작위로 투여하였다. MCAO와 콜라게나아제로 유도된 뇌내출혈을 유도한 후 뇌내출혈의 크기는 헤모글로빈 분석을 통해 측정하였고, 혈관 안정성은 전자현미경을 통한 뇌혈관 기저막의 밀도를 측정하여 조사하였다. 허혈성 뇌출혈 발생 이후 와파린 그룹(12 of 18, 66.7%)에서 플라시보 그룹(5 of 36, 12.9%, $p<0.0001$)과 다비가트란 그룹(6 of 27, 22,2%, $p=0.003$) 보다 뇌내출혈이 더 빈번히 관찰되었다. 그러나 헤모글로빈 수치는 세 그룹에서 차이를 보이지 않았다. 뇌경색 중심부와 뇌경색 주변부의 뇌혈관

기저막 밀도는 와파린 그룹과 다비가트란 그룹에서 차이를 보이지 않았다. 콜라게나아제로 유도된 뇌내출혈 모델에서는 헤모글로빈 수치는 세 그룹에서 비슷하게 측정 되었다.

결과적으로 큰 뇌경색이 유도되었을 때, 와파린을 선처리한 한 그룹보다 다비가트란을 선처리한 그룹에서 뇌내출혈 발생률이 낮았다. 뇌혈관 기저막의 손상은 와파린과 다비가트란을 사용하는 환자 사이에서 뇌내출혈의 위험성을 높이는 주된 원인으로 판단되지 않는다.

핵심되는 말: 뇌경색, 뇌내출혈, 와파린, 다비가트란, 혈종, 뇌혈관 기저막