atherosclerotic peripheral vascular disease and quality of care outcomes in research interdisciplinary working groups. Stroke 2007;38:1655–711.
[24] Takahashi S, Oida K, Fujiwara R, Maeda H, Hayashi S, Takai H, et al. Effect of cilostazol, a cyclic AMP phosphodiesterase inhibitor, on the proliferation of rat aortic smooth muscle cells in culture. J Cardiovasc Pharmacol 1992;20:900–6.
[25] Nonaka Y, Tsuruma K, Shimazawa M, Yoshimura S, Iwama T, Hara H. Cilostazol protects against hemorrhagic transformation in mice transient focal cerebral ischemia-induced brain damage. Neurosci Lett 2009;452:156–61.
[26] Honda F, Imai H, Ishikawa M, Kubota C, Shimizu T, Fukunaga M, et al. Cilostazol attenuates gray and white matter damage in a rodent model of focal cerebral ischemia. Stroke 2006;37:223–8.
[27] Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomized trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ 2002;324:71–86.
[28] Howard PA. Aspirin resistance. Ann Pharmacother 2002;36:1620–4.
[29] Marquardt L, Ruf A, Mansmann U, Winter R, Schuler M, Buggle F, et al. Course of platelet activation markers after ischemic stroke. Stroke 2002;33:2570–4.
[30] Diener HC, Bogousslavsky J, Brass LM, Cimminiello C, Csiba L, Kaste M, et al. Aspirin and clopidogrel compared with clopidogrel alone after recent ischaemic stroke or transient ischaemic attack in high risk patients (MATCH): randomized, double-blind, placebo-controlled trial. Lancet 2004;364:331–7.
[31] Kennedy J, Hill MD, Ryckborst KJ, Eliasziw M, Demchuk AM, Buchan AM. Fast assessment of stroke and transient ischaemic attack to prevent early recurrence (FASTER): a randomized controlled pilot trial. Lancet Neurol 2007;6:961–9.
[32] CAPRIE Steering Committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). Lancet 1996;348:1329–39.
[33] Sacco RL, Diener HC, Yusuf S, Cotton D, Ounpuu S, Lawton WA, et al. PRoFESS study group. Aspirin and extended-release dipyridamole versus clopidogrel for recurrent stroke. N Engl J Med 2008;359:1238–51.
[34] Meyer DM, Albright KC, Allison TA, Grotta JC. LOAD: a pilot study of the safety of loading of aspirin and clopidogrel in acute ischemic stroke and transient ischemic attack. J Stroke Cerebrovasc Dis 2008;17:26–9.
[35] Wilhite DB, Comerota AJ, Schmieder FA, Throm RC, Gaughan JP, Rao AK. Managing PAD with multiple platelet inhibitors: the effect of combination therapy on bleeding time. J Vasc Surg 2003;38:710–3.
[36] Uchiyama S, Demaerschalk BM, Goto S, Shinohara Y, Gotoh F, Stone WM, et al. Stroke prevention by cilostazol in patients with atherothrombosis: meta-analysis of placebo-controlled randomized trials. J Stroke Cerebrovasc Dis 2009;18:482–90.

Dabigatran and Factor Xa Inhibitors for Stroke Prevention in Patients with Nonvalvular Atrial Fibrillation

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Stroke is a major health problem worldwide, and is often fatal or associated with poor long-term outcomes. Atrial fibrillation (AF) is responsible for up to 20% of all strokes; and the risk of stroke in patients with AF increases with age. Although warfarin is well established for the prevention of stroke in patients with AF, it has some limitations, particularly a narrow therapeutic window, variable/unpredictable pharmacokinetic/pharmacodynamic properties, the restriction of vitamin K intake, and the need for regular coagulation monitoring. Therefore, warfarin is underused for stroke prevention in patients with AF. Several anticoagulants that inhibit thrombin or factor Xa have been developed. Dabigatran is a direct thrombin (factor IIa) inhibitor that overcomes many of the limitations associated with warfarin. The recent Randomized Evaluation of Long Term Anticoagulant Therapy study showed the noninferiority of 110 mg and 150 mg dabigatran twice daily, and the superiority of 150 mg dabigatran twice daily versus adjusted-dose warfarin in the prevention of stroke or systemic embolism in patients with nonvalvular AF. In addition, the rate of intracranial hemorrhage was much lower with both doses of dabigatran than with warfarin. Dabigatran was recently approved in Japan for the prevention of ischemic stroke and systemic embolism in patients with nonvalvular AF. Therefore, in this review, we discuss the properties of dabigatran and its clinical efficacy, safety, and positioning in the prevention of stroke. We also discuss precautions for the use of dabigatran and future perspectives with

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Received January 16, 2012; accepted January 22, 2012.

Dr. Uchiyama has received consulting fees and support for travel to meetings, and his institution has received grants from Boehringer Ingelheim, Bayer, and Daiichi-Sankyo. Dr. Ibayashi has received consulting fees and support for travel to meetings from Boehringer Ingelheim and Bayer. Dr. Matsumoto has received consulting fees, and his institution has received grants from Bayer, Boehringer Ingelheim, Daiichi-Sankyo, and Pfizer. Dr. Nagao has received consulting fees and support for travel to meetings, and his institution has received grants from Boehringer Ingelheim, Bayer, and Daiichi-Sankyo. Dr. Nakagawara has received honoraria from Boehringer Ingelheim and Bayer. Dr. Tanahashi has received payment for development of educational presentations from Pfizer Japan, Boehringer Ingelheim, Bayer, and Daiichi-Sankyo. Dr. Tanaka has received consulting fees and support for travel to meetings, and his institution has received grants from Boehringer Ingelheim. Dr. Toyoda and his institution have received grants from Boehringer Ingelheim. Dr. Yasaka and his institution have received grants from Boehringer Ingelheim. Dr. Nagata has no potential conflicts of interest to declare.

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1052-3057/$ - see front matter

doi:10.1016/j.jstrokecerebrovasdis.2012.01.011

a view to reducing the risk of stroke with new oral anticoagulants, including factor Xa inhibitors in AF patients. **Key Words:** Atrial fibrillation—cardioembolic stroke—dabigatran—factor Xa inhibitors—stroke.

Stroke is a major health problem worldwide, and is often fatal or associated with poor long-term outcomes.[1] In Japan, although the mortality rate associated with stroke appears to be decreasing, the overall incidence of stroke has not decreased; in fact, the number of stroke patients has even increased because of the rapid growth of the elderly population.[2-8]

Atrial fibrillation (AF) is a common cause of stroke and is responsible for at least 15% to 20% of all strokes.[9] In a general Japanese population ≥40 years of age, the overall prevalence of AF in men and women was 1.35% and 0.43%, respectively.[10] Among men and women ≥80 years of age, the prevalence of AF was 4.4% and 2.2%, respectively,[10] indicating that the prevalence of AF increases with age.

Considering these findings in the general population, an analysis of the Japanese Stroke Databank (n = 35,414), which included 32,799 patients with acute ischemic stroke and 2,615 patients with transient ischemic attack (TIA), revealed that 21.8% of men and 25.4% of women had AF.[8] As in the general population, the prevalence of AF in stroke patients also increased with age, with 32.3% of men and 35.6% of women ≥80 years of age having AF.[8] Notably, 72.3% of patients with cardioembolic stroke (CES) had AF. It is worrisome that the National Institutes of Health Stroke Scale scores on admission were substantially higher among patients with CES than among those with other types of ischemic stroke (Fig 1A), as were modified Rankin scale scores at discharge (Fig 1B).[8]

Warfarin is recommended by the Japanese Guidelines for the Management of Stroke[11] for stroke prevention in patients with nonvalvular AF (NVAF). Despite these recommendations, according to the Japanese Stroke Databank, warfarin is underused, with only 38.0% of NVAF patients receiving warfarin before the onset of stroke or TIA. In addition, only 12.9% received warfarin despite indications for its use as primary prevention (Fig 1C).[8]

The underuse of warfarin in particular may be related to its limitations, which include a narrow therapeutic window, variable and unpredictable pharmacokinetic and pharmacodynamic properties, interactions with other drugs and vitamin K-rich foods, a slow onset and offset of action, and the need for regular anticoagulation monitoring and dose adjustments (Table 1). Another factor that may limit the use of warfarin is the higher risk of intracranial hemorrhage (ICH) among Asian patients with AF compared with white patients with AF.[12]

Several anticoagulants with novel pharmacologic targets have recently been approved for clinical use or are currently under clinical evaluation. These novel drugs include direct thrombin inhibitors (eg, dabigatran) and factor Xa inhibitors (eg, rivaroxaban, apixaban, and

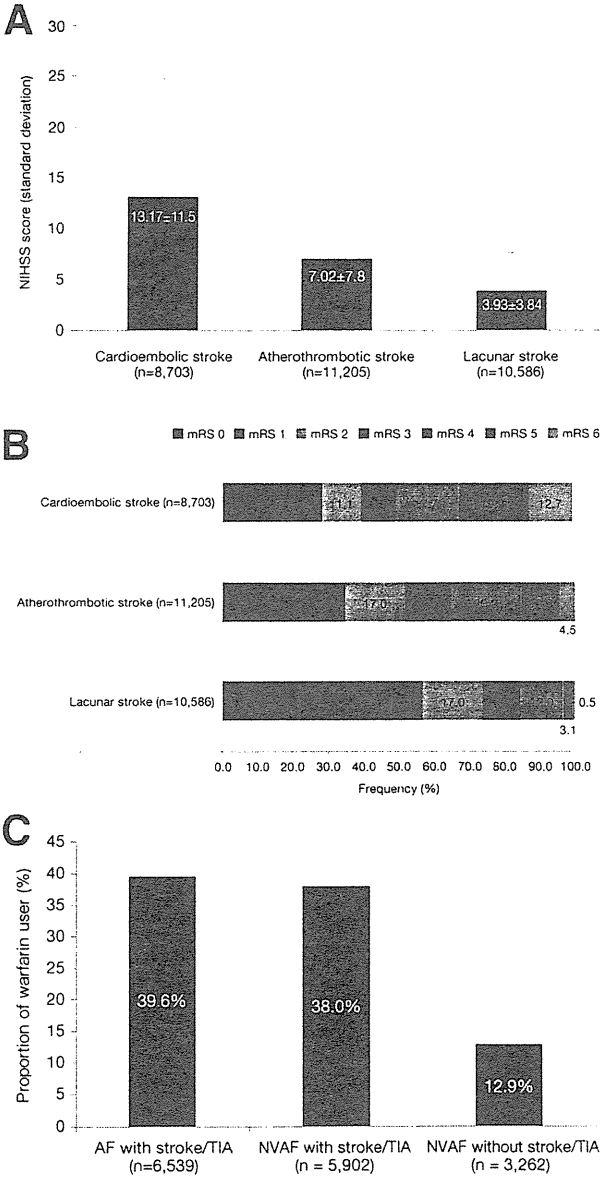

Figure 1. *Results from the Japanese Stroke Databank 2009. National Institutes of Health Stroke Scale (NIHSS) scores (means ± SD) on admission according to subtype of ischemic stroke (A). Proportions of patients with each modified Rankin scale (mRS) score at discharge according to subtype of ischemic stroke (B). Proportions of warfarin use in patients with atrial fibrillation or nonvalvular atrial fibrilliation both with and without stroke or TIA (C). Data from Fukuda et al.[8] Abbreviations: AF, atrial fibrillation; NVAF, nonvalvular atrial fibrillation; TIA, transient ischemic attack.*

Table 1. *Limitations of warfarin use*

Narrow therapeutic window
Variable and unpredictable pharmacokinetic and pharmacodynamic properties
Interactions with other drugs and foods rich in vitamin K
Slow onset and offset of action
Need for regular anticoagulation monitoring and dose adjustments
High incidence of intracranial bleeding, especially among Asian patients

edoxaban; Table 2).[13] Their pharmacologic targets in the coagulation cascade are summarized in Fig 2. Dabigatran, rivaroxaban, apixaban, and edoxaban are orally active drugs administered either once daily (rivaroxaban and edoxaban) or twice daily (dabigatran and apixaban). Although the half-lives of these drugs vary, they reach maximum concentrations within approximately 1 to 4 hours.

Dabigatran was recently approved in Japan for the prevention of ischemic stroke and/or systemic embolism in patients with NVAF. Considering the results of recently published and ongoing trials, such as Rivaroxaban-once Daily, Oral, Direct Factor Xa Inhibition Compared with Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation (ROCKET-AF),[14] Apixaban Versus Acetylsalicylic Acid to Prevent Strokes (AVERROES),[15] Apixaban for the Prevention of Stroke in Subjects With Atrial Fibrillation (ARISTOTLE),[16]and Global Study to Assess the Safety and Effectiveness of DU-176b Versus Standard Practice of Dosing with Warfarin in Patients with Atrial Fibrillation (ENGAGE-AF; ongoing, NCT00781391), it is likely that these factor Xa inhibitors for the prevention of ischemic stroke and systemic embolism will also be applicable.

Considering the current status of these drugs, the objective of this review is to discuss opportunities to prevent ischemic stroke and systemic embolism in patients with AF, focusing on dabigatran, and therefore to provide a practical update for clinicians treating these patients.

Pharmacologic Characteristics of Dabigatran

Thrombin is a plasma serine protease that plays a major role in coagulation and hemostasis (Fig 2). In vivo, thrombin is produced by the cleavage of prothrombin and catalyzes the conversion of fibrinogen to fibrin, which promotes thrombus formation. Therefore, thrombin was selected as a therapeutic target because it is a key molecule that plays an essential role in the coagulation cascade. Accordingly, dabigatran, by targeting thrombin, significantly disrupts the coagulation cascade and prevents the formation of fibrin.

In terms of its mechanism of action, dabigatran competitively inhibits thrombin in a concentration-dependent manner.[17] Its binding to thrombin is highly selective and occurs rapidly, but is reversible. The general pharmacologic characteristics of dabigatran, a nonpeptide direct thrombin (factor IIa) inhibitor,[18] are summarized in Table 2. Briefly, after oral administration, dabigatran etexilate is absorbed via the gastrointestinal (GI) tract and rapidly hydrolyzed by esterase to its active form, dabigatran.[19] Dabigatran reaches a peak plasma concentration approximately 0.5 to 2 hours after administration. It has a terminal half-life of approximately 12 to 17 hours[20] and an absolute bioavailability of 6.5%. Approximately 80% of dabigatran is excreted by the kidney.[18,19] Dabigatran is not metabolized by nor does it affect the activity of the cytochrome P450 system.[19] As a result, it has few drug interactions and does not necessitate diets with low vitamin K content.[20] However, dabigatran etexilate is a substrate of P-glycoprotein, and its absorption is influenced by a number of P-glycoprotein inhibitors and inducers.[21]

Table 2. *Profiles of novel oral anticoagulants**

	Dabigatran	Rivaroxaban	Apixaban	Edoxaban
Target	Thrombin	Xa	Xa	Xa
Administration	Twice daily	Once daily	Twice daily	Once daily
Prodrug	Yes	No	No	No
Half-life (hrs)	12-14	9-13	8-15	6-11
t_{max} (hrs)	0.5-2	2-4	1-4	1-1.5
Bioavailability	6.5% (humans)	67-86% (animals)	49% (humans)	60% (animals)
Protein binding	35%	92-95%	87%	40-59%
Renal excretion	80%	33%	25%	35-39%
Phase III trials (status)	RE-LY (published)[22,23]	ROCKET-AF (published)[14]	AVERROES (published)[15]	ENGAGE-AF (ongoing; NCT00781391)
		J-ROCKET (completed; NCT00494871)	ARISTOTLE (published)[16]	

*Data from Ogawa et al.[13]

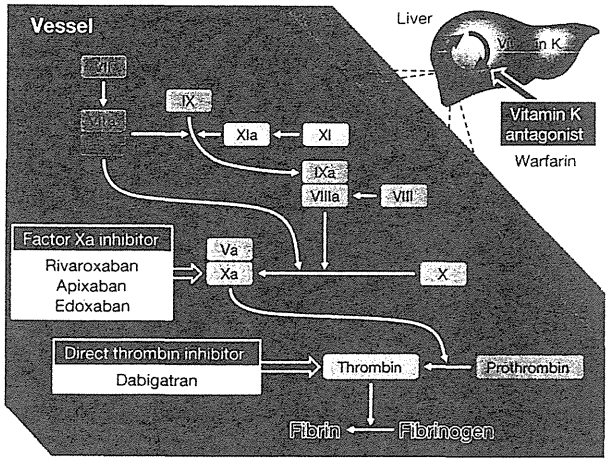

Figure 2. *Site of action of oral anticoagulants.*

Dabigatran exhibits dose-proportional and near-linear increases in activated partial thromboplastin time (aPTT), at least with the concentrations of dabigatran applied in clinical practice, prothrombin time (PT), thrombin time (TT), and ecarin clotting time (ECT).[20] In addition, the variability in pharmacokinetic and pharmacodynamic properties was generally low or moderate, indicating predictable pharmacokinetic and pharmacodynamic characteristics.

Clinical Efficacy of Dabigatran in Patients with Atrial Fibrillation

Overall Efficacy

The clinical efficacy and safety of dabigatran were assessed in the Randomized Evaluation of Long Term Anticoagulant Therapy (RE-LY) study, in which 18,113 patients with NVAF were treated with either 110 mg or 150 mg dabigatran twice daily, or with adjusted-dose warfarin for approximately 2 years. The primary endpoint of the study was stroke or systemic embolism.[22] The overall rate of stroke and systemic embolism was 1.71% in patients treated with warfarin versus 1.54% for 110 mg dabigatran (relative risk vs warfarin 0.90; 95% confidence interval [CI] 0.74-1.10; $P < .001$ for noninferiority and $P = .30$ for superiority) and 1.11% for 150 mg dabigatran (relative risk vs warfarin 0.65; 95% CI 0.52-0.8; $P < .001$ for noninferiority and $P < .001$ for superiority; Fig 3).[22,23] Collectively, these results showed that both doses of dabigatran were noninferior to warfarin, and that 150 mg dabigatran showed superiority to warfarin for the prevention of stroke and systemic embolism in patients with NVAF. The rates of major bleeding, hemorrhagic stroke, and mortality tended to be lower in patients treated with 110 mg or 150 mg dabigatran than in patients treated with warfarin.[22,23] As shown in Fig 4, dabigatran also significantly reduced the risk of intracranial hemorrhage, with risk reductions of 59% with 150 mg dabigatran and 70% with 110 mg dabigatran.

Efficacy in Patients with Previous Stroke or TIA

Because the RE-LY study also included patients with previous stroke or TIA, the investigators compared the efficacy of dabigatran with that of warfarin in this subgroup.[24] The results of this subanalysis showed the same trend as those seen in the overall study population; the risk reductions for stroke or systemic embolism with 110 mg and 150 mg dabigatran relative to warfarin were 16% and 25%, respectively (Fig 3), although these differences were not statistically significant because of the small number of patients.[24] On the other hand, as shown in Fig 4, both doses of dabigatran significantly reduced the risk of intracranial hemorrhage in patients with previous stroke or TIA, by 80% and 59% for 110 mg and 150 mg dabigatran, respectively, compared with warfarin.[24]

Positioning of Dabigatran

The Congestive heart failure, Hypertension, Age, Diabetes mellitus, and Prior Stroke or TIA ($CHADS_2$) score is a tool to estimate the risk of stroke in patients with AF and help determine whether anticoagulant therapy is needed.[25] To date, warfarin has been used in patients at higher risk ($CHADS_2$ score ≥ 2). Among subgroups of patients in the RE-LY study stratified by $CHADS_2$ scores of 0 to 1, 2, and 3 to 6,[26] dabigatran was associated with lower rates of stroke, systemic embolism, and major bleeding in all stratified subgroups, indicating that dabigatran is applicable to all of these risk groups.

In summary, the results of the RE-LY study indicate that, overall, dabigatran is associated with a reduced risk of stroke or systemic embolism compared with warfarin. Therefore, as shown in Fig 5, dabigatran is strongly recommended for patients with $CHADS_2$ scores of 1.[27] For patients with $CHADS_2$ scores ≥ 2, dabigatran and warfarin are equally appropriate, and dabigatran is preferred considering the results of the RE-LY study.

In terms of dosage of dabigatran for the prevention of stroke and systemic embolism in patients with AF, 150 mg is recommended, while 110 mg could be considered in patients with higher dabigatran concentrations or patients who are at an increased risk for bleeding.[27]

Precautions for Usage and Contraindications of Dabigatran

In the RE-LY study, the rate of major bleeding was lower than or similar to that in warfarin-treated patients, and the rate of intracranial bleeding was much lower in dabigatran-treated patients than in warfarin-treated patients.[22,23] However, considering that thrombin is a key molecule in the coagulation cascade, patients using dabigatran should be aware that increased bleeding is possible with this drug. Therefore, dabigatran should be used with care taken to reduce the risk of bleeding. The

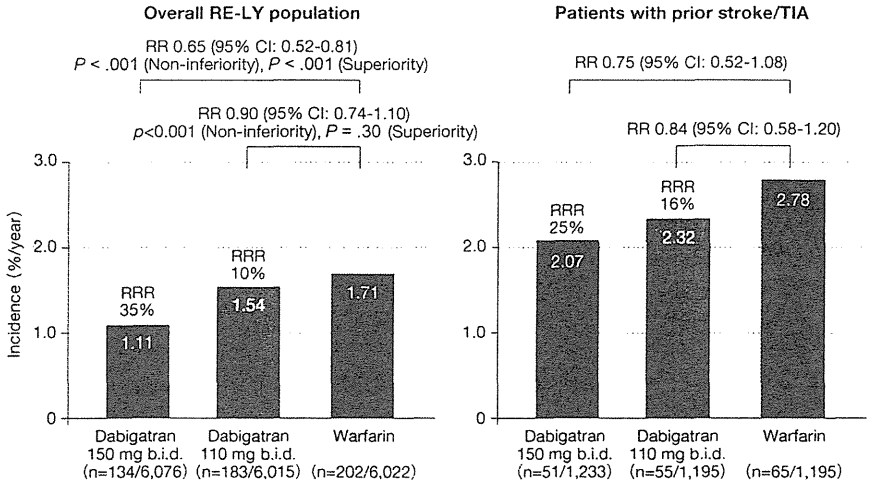

Figure 3. *Effects of dabigatran and warfarin on the incidence of stroke or systemic embolism in the Randomized Evaluation of Long-term Anticoagulant Therapy study in all patients and in patients with previous stroke or transient ischemic attack. Data from Connolly et al,[23] and Diener et al.[24]*

precautions and contraindications to dabigatran are summarized in Table 3.

There was a significant treatment by age interaction, such that dabigatran 110 mg twice daily was associated with a lower risk of major bleeding in patients <75 years of age and a similar risk in those ≥75 years of age compared with warfarin, whereas dabigatran 150 mg twice daily was associated with a lower risk of major bleeding in those <75 years of age and a trend toward higher risk of major bleeding in those ≥75 years of age compared with warfarin.[28]

On the other hand, the incidence of GI bleeding, particularly lower GI bleeding, is higher among dabigatran-treated patients than warfarin-treated patients.[28] Eikelboom et al[29] proposed several mechanisms to describe the increase in major GI bleeding, particularly among patients >75 years of age. First, they suggested that because dabigatran has a low bioavailability after oral ingestion, the metabolism of dabigatran etexilate may lead to higher concentrations of the active drug during transit of the GI tract, leading to local effects. In addition, elderly individuals are more likely to have GI tract pathologies, increasing their risk for bleeding.[29] In contrast, warfarin is activated after hepatic metabolism; therefore, unabsorbed warfarin cannot be activated in the GI tract, reducing the risk for GI bleeding.

Considering the increasing use of antiplatelet drugs, such as aspirin and clopidogrel, the efficacy and safety of dabigatran in combination with these drugs is an important topic of research in terms of stroke prevention and risk of bleeding events. When used concomitantly with aspirin, dabigatran showed good efficacy in terms of stroke or systemic embolism, similar to that of dabigatran alone.[22] However, because the concomitant use of aspirin increases the risk of major bleeding in patients treated with dabigatran or warfarin, precautions for coadministration are necessary.

Treatment of Bleeding Complications

Because bleeding complications are the main concern of all anticoagulant drugs, patients should be aware of the risks and physicians should know how to treat such complications. Table 4 summarizes the main interventions that should be considered in anticoagulant-treated patients who experience bleeding complications.

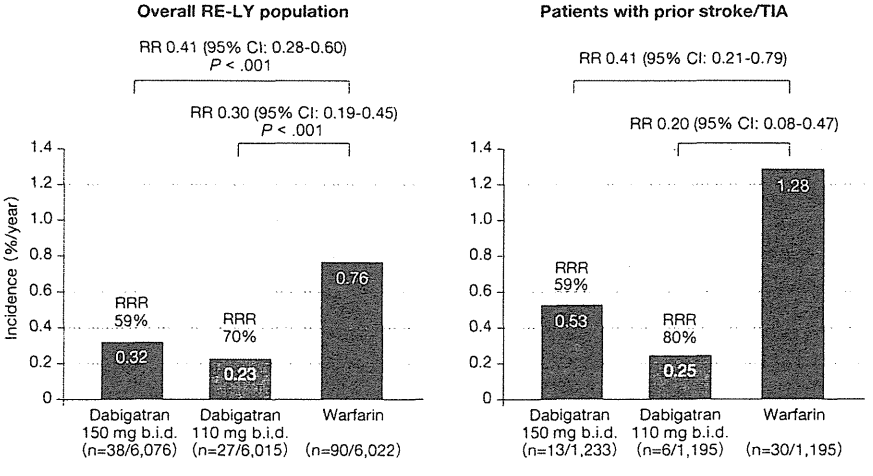

Figure 4. *Incidence of intracranial hemorrhage in the Randomized Evaluation of Long-term Anticoagulant Therapy study in all patients and in patients with previous stroke or transient ischemic attack. Intracranial hemorrhage includes hemorrhagic stroke, subarachnoid hemorrhage, and subdural hematoma. Data from Connolly et al,[23] and Diener et al.[24]*

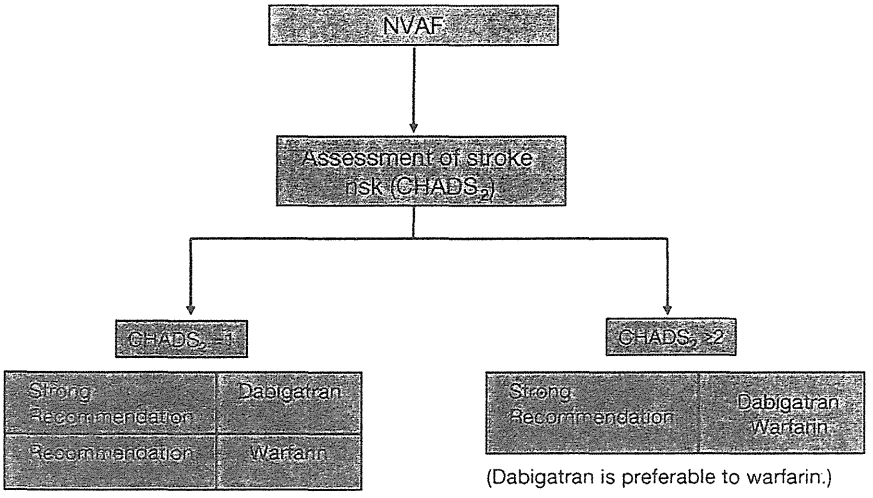

Figure 5. *Recommendation of dabigatran for the prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation.*

It is important to treat the bleed as promptly and efficiently as possible. Because dabigatran is renally excreted, appropriate intravenous infusion and induction of diuresis are beneficial. For major bleeds, it is important to halt its administration and consider performing gastric lavage or oral administration of activated charcoal if the bleed occurs within 2 hours of dabigatran administration. It is also important to support the circulatory system by supplementing endogenous procoagulant factors, such as fresh frozen plasma (FFP), factor IX complex (prothrombin complex concentrate [PCC]), which contains factors II, VII, IX, and X, or recombinant factor VII. In preclinical studies, it was found that PCC or recombinant factor VII inhibited the prolongation of bleeding time after the administration of dabigatran to rats.[30,31] On the other hand, in a clinical study of healthy volunteers, it was reported that PCC did not reverse the anticoagulant effects of dabigatran on aPTT, ECT, or TT,[32] but there has been no report in which bleeding time was evaluated. It is therefore necessary to verify the effects of PCC, recombinant factor VII, and FFP on bleeding during treatment with the new anticoagulants with accumulating clinical experience. Hemodialysis to remove dabigatran or blood transfusion may also be necessary, depending on the severity. Finally, as discussed in greater detail below, the development of antibodies capable of neutralizing dabigatran may also offer an important option for patients with severe bleeding.

Future Perspectives

Considering that warfarin requires several days before the onset of its anticoagulant effects—whereas the effects of dabigatran are apparent much sooner—it is possible that the duration of hospitalization for patients with acute AF-related stroke could be shortened by using dabigatran instead of warfarin. There are currently no data from the

Table 3. *Precautions and contraindications to dabigatran**

Precautions

- Consider administering dabigatran at 110 mg twice daily and carefully administer this product as the blood dabigatran concentrations may increase in the following patients:
  - Patients with moderate renal impairment (with creatinine clearance 30-50 mL/min)
  - Patients under treatment with an oral P-glycoprotein inhibitor
- Dabigatran should be administered with care, with consideration of 110 mg twice daily dosing, in the following patients at high risk of hemorrhage:
  - Patients ≥70 years of age
  - Patients with a history of gastrointestinal hemorrhage

Contraindications

- Patients with a history of hypersensitivity to the active ingredients of this product
- Patients with severe renal impairment (creatinine clearance <30 mL/min), including those undergoing dialysis
- Patients with hemorrhagic symptoms, hemorrhagic diathesis, or spontaneous or pharmacologic impairment of hemostasis
- Patients with organic lesions associated with risk of clinically significant hemorrhage, including hemorrhagic stroke, within the last 6 months
- Patients with an indwelling spinal or epidural catheter and during the first hour after removal
- Patients under concomitant treatment with itraconazole (oral)

*Data from the Prazaxa (dabigatran etexilate mesylate) package insert.[45]

Table 4. *Treatment of major bleeding complications**

The following interventions must be performed:
- Stop oral medications†
- Mechanical compression and/or surgical interventions. Even after hemostasis, interventions to prevent the recurrence of hemorrhage are necessary
- Maintain circulating blood volume and blood pressure by blood transfusion, for example, and provide treatment to induce diuresis
- For intracerebral hemorrhage and subarachnoid hemorrhage, adequate treatment to suppress blood pressure should be provided

The following interventions should be considered depending on the situation:
- Administration of fresh frozen plasma,‡ factor IX complex‡, §, ¶ (to improve hemostatic function by factor II contained), or recombinant factor VII‡, § (to improve overall hemostatic function)
- Gastric lavage‡ or oral administration of activated charcoal‡ (within 2 hours of oral administration)
- Hemodialysis‡
- Blood transfusion

*Data from van Ryn et al.[46]

†Temporary or permanent withdrawal of dabigatran etexilate may increase the risk of thromboembolism. Therefore, in such cases, switching to another anticoagulant (eg, heparin) may be necessary.

‡Insufficient clinical data.

§Not covered by national health insurance.

¶Factor IX complex contains coagulation factors II, VII, IX, and X.

RE-LY study to confirm this hypothesis, so additional studies are needed to examine this possibility.

Another topic of research is the cost effectiveness of dabigatran relative to that of warfarin. As with many new drugs, dabigatran is more expensive than the established drug (warfarin). Therefore, physicians may be less willing to use this drug considering its cost. However, the cost of these drugs must be weighed against the cost of treating stroke and systemic embolism. Considering the risk reduction profile of dabigatran, it appears to show better cost effectiveness than warfarin in Japan[33] and in other countries.[34-36]

To date, there has been no antidote for dabigatran. However, another approach currently under evaluation is the use of antibodies capable of neutralizing dabigatran. In fact, van Ryn et al[37] recently reported that monoclonal antibodies completely inhibited the anticoagulant activity of dabigatran in human plasma and whole blood in vitro and in rats in vivo.[37] Experimental and clinical studies are now needed to confirm the efficacy and safety of this approach before antibody-based inhibition of dabigatran can be introduced and used in the management of patients with major bleeding complications.

Recombinant tissue plasminogen activator (t-PA) is an important tool used in acute stroke treatment to achieve adequate thrombolysis. In the United States, for example, its use after ischemic stroke has doubled in the last 5 years.[38] t-PA has also been approved in Japan, having shown good efficacy in clinical and postmarketing studies.[39-42] However, there is currently no clear guide for the use of t-PA in patients treated with dabigatran; such a guideline is urgently required to help improve the treatment of stroke.

The results of a subanalysis of Japanese patients in the RE-LY trial showed similar efficacy and safety of dabigatran to those seen in the overall study population.[43] However, the number of patients in that report was relatively small. In Japan, on the other hand, cases of hemorrhage-related death and serious hemorrhage have been reported in the Safety News Bulletin.[44] Given that some of the patients who died were contraindicated for dabigatran, it is important to comply with the package insert.[45] In addition, dabigatran should be used with caution in the elderly and patients with renal dysfunction. However, considering the estimated number of patients who use this product following its launch in Japan, it seems unlikely that the numbers of fatalities and cases with hemorrhage will exceed the risk highlighted in the RE-LY study. Additional clinical studies, including postmarketing surveillance studies in Japanese patients, are planned to provide further confirmation of the efficacy and safety of dabigatran.

Summary

This review has summarized the research supporting the use of dabigatran for the prevention of stroke and systemic embolism in patients with NVAF. Both doses tested in the RE-LY study (110 mg and 150 mg twice daily) were effective and noninferior to warfarin, while 150 mg dabigatran was superior to warfarin. Notably, both doses of dabigatran were associated with a lower risk of intracranial bleeding compared with warfarin. Dabigatran is also effective for secondary prevention, with a lower risk of cerebral hemorrhage. Despite this, further accumulation of clinical data may be needed to confirm the efficacy and safety of dabigatran.

Additional research into its effect on hospital stay after acute ischemic stroke and potential for therapy with t-PA is necessary. The latter is particularly important because there is no clear guideline for the use of t-PA in patients treated with dabigatran. In the future, it will be important for physicians to discern which patients treated with dabigatran should also be treated with t-PA.

Finally, several factor Xa inhibitors will be approved in the near future with similar indications to those of dabigatran and warfarin. Considering this situation, it will be necessary to establish a consensus on how and when to use each of these drugs for the benefit of patients with AF.

References

1. Redon J, Olsen MH, Cooper RS, et al. Stroke mortality and trends from 1990 to 2006 in 39 countries from Europe and Central Asia: Implications for control of high blood pressure. Eur Heart J 2011;32:1424-1431.
2. Imano H, Kitamura A, Sato S, et al. Trends for blood pressure and its contribution to stroke incidence in the middle-aged Japanese population: The Circulatory Risk in Communities Study (CIRCS). Stroke 2009;40: 1571-1577.
3. Kitamura A, Sato S, Kiyama M, et al. Trends in the incidence of coronary heart disease and stroke and their risk factors in Japan, 1964 to 2003: The Akita-Osaka study. J Am Coll Cardiol 2008;52:71-79.
4. Kubo M, Hata J, Doi Y, et al. Secular trends in the incidence of and risk factors for ischemic stroke and its subtypes in Japanese population. Circulation 2008;118: 2672-2678.
5. Kubo M, Kiyohara Y, Kato I, et al. Trends in the incidence, mortality, and survival rate of cardiovascular disease in a Japanese community: The Hisayama study. Stroke 2003;34:2349-2354.
6. Ma E, Takahashi H, Mizuno A, et al. Stratified age-period-cohort analysis of stroke mortality in Japan, 1960 to 2000. J Stroke Cerebrovasc Dis 2007;16:91-102.
7. Kita Y, Turin TC, Ichikawa M, et al. Trend of stroke incidence in a Japanese population: Takashima stroke registry, 1990-2001. Int J Stroke 2009;4:241-249.
8. Fukuda J, Ohkushi Y, Kobayashi S. Japanese stroke databank, 2009. Part 2: Evidence for diagnosis and treatment of stroke. Tokyo: Nakayama Shoten Co., Ltd, 2009.
9. Lloyd-Jones D, Adams RJ, Brown TM, et al. Heart disease and stroke statistics. 2010 update: A report from the American Heart Association. Circulation 2010;121: e46-e215.
10. Inoue H, Fujiki A, Origasa H, et al. Prevalence of atrial fibrillation in the general population of Japan: An analysis based on periodic health examination. Int J Cardiol 2009; 137:102-107.
11. Shinohara Y, Fujinaka T, Yanagihara T, et al. Japanese guidelines for the management of stroke. J Stroke Cerebrovasc Dis 2011;20(Suppl 1):S1-S209.
12. Shen AY, Yao JF, Brar SS, et al. Racial/ethnic differences in the risk of intracranial hemorrhage among patients with atrial fibrillation. J Am Coll Cardiol 2007; 50:309-315.
13. Ogawa S, Koretsune Y, Yasaka M, et al. Antrithrombotic therapy in atrial fibrillation:Evaluation and positioning of new oral anticoagulant agents. Circ J 2011;75: 1539-1547.
14. Patel MR, Mahaffey KW, Garg J, et al. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. N Engl J Med 2011;365:883-891.
15. Connolly SJ, Eikelboom J, Joyner C, et al. Apixaban in patients with atrial fibrillation. N Engl J Med 2011;364: 806-817.
16. Granger CB, Alexander JH, McMurray JJ, et al. Apixaban versus warfarin in patients with atrial fibrillation. N Engl J Med 2011;365:981-992.
17. Wienen W, Stassen JM, Priepke H, et al. In-vitro profile and ex-vivo anticoagulant activity of the direct thrombin inhibitor dabigatran and its orally active prodrug, dabigatran etexilate. Thromb Haemost 2007;98:155-162.
18. Stangier J. Clinical pharmacokinetics and pharmacodynamics of the oral direct thrombin inhibitor dabigatran etexilate. Clin Pharmacokinet 2008;47:285-295.
19. Blech S, Ebner T, Ludwig-Schwellinger E, et al. The metabolism and disposition of the oral direct thrombin inhibitor, dabigatran, in humans. Drug Metab Dispos 2008; 36:386-399.
20. Stangier J, Rathgen K, Stähle H, et al. The pharmacokinetics, pharmacodynamics and tolerability of dabigatran etexilate, a new oral direct thrombin inhibitor, in healthy male subjects. Br J Clin Pharmacol 2007;64: 292-303.
21. Bendel SD, Bona R, Baker WI. Dabigatran: An oral direct thrombin inhibitor for use in atrial fibrillation. Adv Ther 2011;28:460-472.
22. Connolly SJ, Ezekowitz MD, Yusuf S, et al. Dabigatran versus warfarin in patients with atrial fibrillation. N Engl J Med 2009;361:1139-1151.
23. Connolly SJ, Ezekowitz MD, Yusuf S, et al. Newly identified events in the RE-LY trial. N Engl J Med 2010; 363:1875-1876.
24. Diener HC, Connolly SJ, Ezekowitz MD, et al. Dabigatran compared with warfarin in patients with atrial fibrillation and previous transient ischaemic attack or stroke: A subgroup analysis of the RE-LY trial. Lancet Neurol 2010; 9:1157-1163.
25. Gage BF, Waterman AD, Shannon W, et al. Validation of clinical classification schemes for predicting stroke: Results from the National Registry of Atrial Fibrillation. JAMA 2001;285:2864-2870.
26. Oldgren J, Alings M, Darius H, et al. Risks for stroke, bleeding, and death in patients with atrial fibrillation receiving dabigatran or warfarin in relation to the CHADS2 score: A subgroup analysis of the RE-LY trial. Ann Int Med 2011;155:660-667.
27. Ogawa S, Hori M. Urgent statement on antithrombotic therapy of atrial fibrillation. Circ J 2011;75:2719-2721.
28. Eikelboom JW, Wallentin L, Connolly SJ, et al. Risk of bleeding with 2 doses of dabigatran compared with warfarin in older and younger patients with atrial fibrillation: An analysis of the Randomized Evaluation of Long-term Anticoagulant Therapy (RE-LY) trial. Circulation 2011; 123:2363-2372.
29. Strate LL. Lower GI bleeding: Epidemiology and diagnosis. Gastroenterol Clin North Am 2005;34:643-664.
30. van Ryn J, Ruehl D, Priepke H, et al. Reversibility of the anticoagulant effect of high doses of the direct thrombin inhibitor, dabigatran, by recombinant factor VIIa or activated prothrombin complex concentrate. Haematologica 2008;93(Suppl 1):148.
31. van Ryn J, Schurer J, Kink-Eiband M, et al. The successful reversal of dabigatran-induced bleeding by coagulation factor concentrates in a rat tail bleeding model do not correlate with ex vivo markers of anticoagulation.

Blood (ASH Annual Meeting Abstracts) 2011;118:2316 [abstr].
32. Eerenberg ES, Kamphuisen PW, Sijpkens MK, et al. Reversal of rivaroxaban and dabigatran by prothrombin complex concentrate: A randomized, placebo-controlled, crossover study in healthy subjects. Circulation 2011; 124:1573-1579.
33. Hori M, Koretsune Y, Yasaka M. Evaluation of the medical economic potential of dabigatran etexilate toward stroke prevention for non-valvular atrial fibrillation (NVAF) [in Japanese]. Pharma Medica 2011;29:151-164.
34. Freeman JV, Zhu RP, Owens DK, et al. Cost-effectiveness of dabigatran compared with warfarin for stroke prevention in atrial fibrillation. Ann Intern Med 2011;154:1-11.
35. Sorensen SV, Kansal AR, Connolly S, et al. Cost-effectiveness of dabigatran etexilate for the prevention of stroke and systemic embolism in atrial fibrillation: A Canadian payer perspective. Thromb Haemost 2011;105:908-919.
36. Shah SV, Gage BF. Cost-effectiveness of dabigatran for stroke prophylaxis in atrial fibrillation. Circulation 2011; 123:2562-2570.
37. van Ryn J, Litzenburger T, Waterman A, et al. Dabigatran anticoagulant activity is neutralized by an antibody selective to dabigatran in vitro and in vivo models. J Am Coll Cardiol 2011;57(14 Suppl):E1130.
38. Adeoye O, Hornung R, Khatri P, et al. Recombinant tissue-type plasminogen activator use for ischemic stroke in the United States: A doubling of treatment rates over the course of 5 years. Stroke 2011;42:1952-1955.
39. Mori E, Minematsu K, Nakagawara J, et al. Effects of 0.6 mg/kg intravenous alteplase on vascular and clinical outcomes in middle cerebral artery occlusion: Japan Alteplase Clinical Trial II (J-ACT II). Stroke 2010;41:461-465.
40. Nakagawara J, Minematsu K, Okada Y, et al. Thrombolysis with 0.6 mg/kg intravenous alteplase for acute ischemic stroke in routine clinical practice: The Japan post-Marketing Alteplase Registration Study (J-MARS). Stroke 2010;41:1984-1989.
41. Toyoda K, Koga M, Naganuma M, et al. Routine use of intravenous low-dose recombinant tissue plasminogen activator in Japanese patients: General outcomes and prognostic factors from the SAMURAI register. Stroke 2009;40:3591-3595.
42. Yamaguchi T, Mori E, Minematsu K, et al. Alteplase at 0.6 mg/kg for acute ischemic stroke within 3 hours of onset: Japan Alteplase Clinical Trial (J-ACT). Stroke 2006; 37:1810-1815.
43. Hori M, Connolly SJ, Ezekowitz MD, et al. Efficacy and safety of dabigatran vs. warfarin in patients with atrial fibrillation—Sub-analysis in Japanese population in RE-LY trial. Circ J 2011;75:800-805.
44. Boehringer Ingelheim. Safety news bulletin. August 2011. Available at: http://www.bij-kusuri.jp/information/attach/pdf/blue_letter_201108.pdf.
45. Prazaxa (dabigatran etexilate mesylate) [package insert]. Tokyo, Japan: Nippon Boehringer Ingelheim Co, Ltd, 2011 [in Japanese].
46. van Ryn J, Stangier J, Haertter S, et al. Dabigatran etexilate—A novel, reversible, oral direct thrombin inhibitor: Interpretation of coagulation assays and reversal of anticoagulant activity. Thromb Haemost 2010;103: 1116-1127.

HEART's Selection 1

新時代に入った抗血栓治療

新規経口抗凝固薬up to date

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はじめに

急激な高齢化社会の進行に伴って，心房細動による脳塞栓症が増加している．心房細動患者の脳塞栓症予防には抗凝固療法の適応があるが，わが国では経口投与可能な唯一の抗凝固薬としてワルファリンが60年以上もの間用いられてきた．しかしながら，ワルファリンには血液凝固モニター，ビタミンK摂取制限，他剤との相互作用のチェックの必要性などの不便さがあり，脳出血への危惧と相まって，本来適応となるべき症例に投与されないことも多かった．最近，これらワルファリンの不便さをすべて解消する経口投与可能な抗凝固薬として，直接的トロンビン阻害薬や凝固Xa因子阻害薬が開発され，心房細動患者を対象として大規模なワルファリンとの比較試験が行われた．その中で直接的トロンビン阻害薬ダビガトランは臨床試験成績に基づき，最近，日本でも保険適用が承認され，多くの症例に用いられるようになった．また，Xa因子阻害薬もリバロキサバンとアピキサバンの試験成績が発表された．

新規抗凝固薬の作用機序

ワルファリンは，ビタミンK依存性の複数の凝固因子の合成を抑制することによりトロンビンの生成を抑制する間接的トロンビン阻害薬であり，その肝臓でのcytochrome(CYP)代謝が遺伝子の制御下にあるため，効果に個人差が大きく，食事や薬剤の影響を受けやすく，治療域が狭いため，ビタミンK摂取制限，他剤との相互作用のチェック，時間と経費を要する血液凝固モニターが必要となる．これに対して，新規抗凝固薬は血液凝固の特異的な段階に作用する血液凝固因子に選択的に作用して，その機能を阻害する分子標的薬であることから，用量反応性に優れ，抗凝固活性の個人差が少なく，ビタミンK摂取制限の必要がなく，薬物相互作用がほとんどなく，血液凝固モニターを必要としない[1)2)]．

直接的トロンビン阻害薬

経口の直接的トロンビン阻害薬の中で臨床開発が継続されたのはダビガトランのみであり[3)]，心房細動患者においてワルファリン〔国際標準比(international normalized ratio；INR) 2～3〕との有効性と安全性を比較する第3相臨床試験(Randomized Evaluation of Long term anticoagulant therapY；RE-LY)が行われた[4)]．

RE-LY試験の対象患者は，少なくとも1つの危険因子〔脳卒中，一過性脳虚血発作(transient ischemic attack；TIA)または全身塞栓症の既往，左室機能不全，75歳以上，高血圧，冠動脈疾患，糖尿病のいずれかを有する65歳以上〕がある心房細動患者18,113例であり，このうち日本人患者が326例含まれていたが，これらの症例を3群に無作為割り付けし，二重盲検下でダビガトラン110mg 1日2回，または，ダビガトラン150mg 1日2回を投与するか，非盲検下でワルファリン(INRの目標値2.0～3.0，日本人のみINR 2.0～2.6)を投与し，1～3年間(平均2年間)追跡調査した[4)]．

本研究の目的はワルファリンに対するダビガトランの非劣性を証明することにあったが，結果はいずれの用量のダビガトランも非劣性が証明されたのみならず，高用量の有効性と低用量の安全性はワルファリンを有意に上回っていた．すなわち，脳卒中また

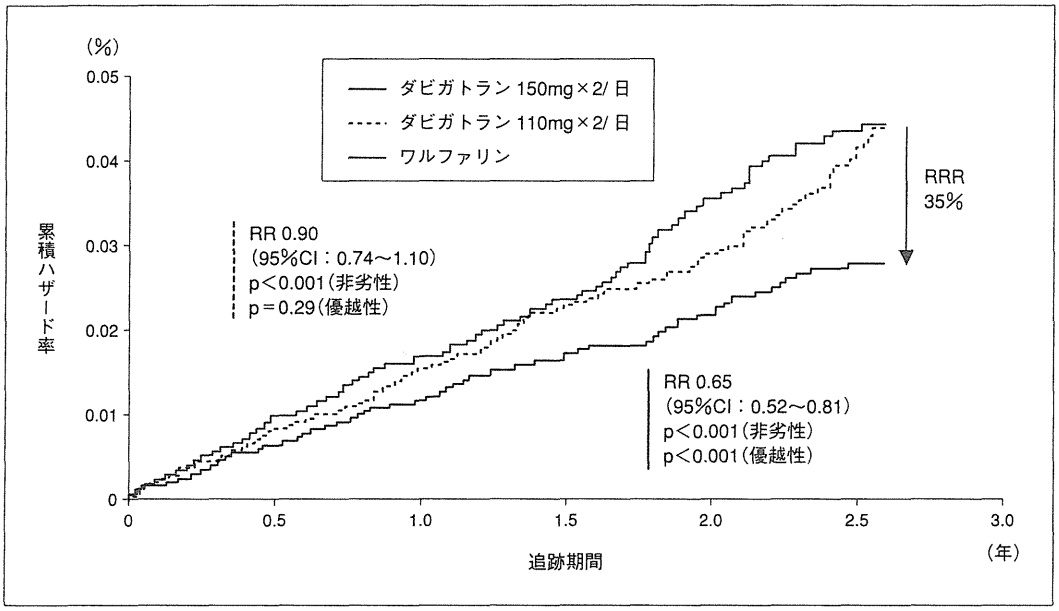

図1
RE-LYの成績主要評価項目－脳卒中または全身性塞栓症－
（文献4より改変引用）

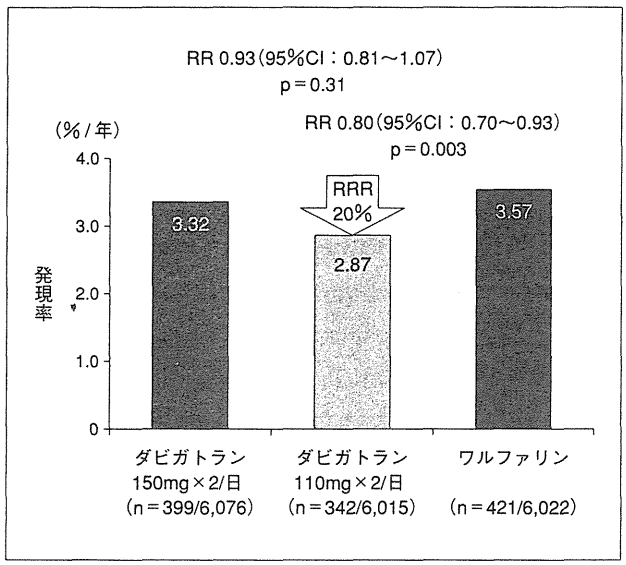

図2　RE-LYの成績－大出血－
（文献4より改変引用）

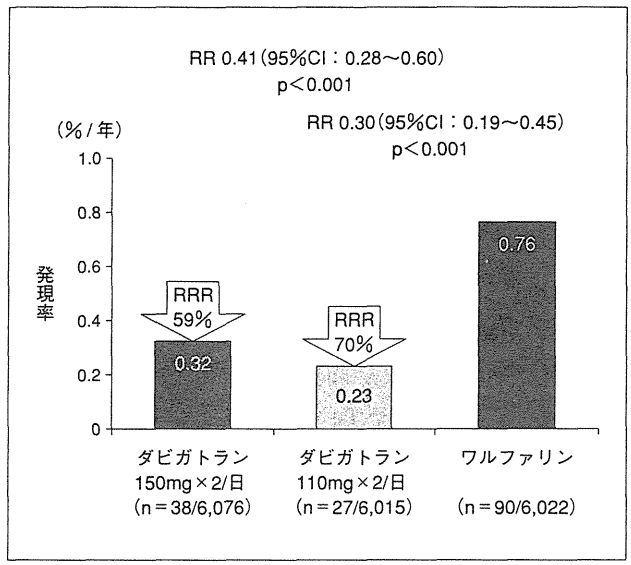

図3　RE-LYの成績－頭蓋内出血の発現率－
（文献4より改変引用）

は全身塞栓症の発症率はワルファリン投与群(1.69%/年)よりダビガトラン150mg 2回投与群で有意に少なく，ダビガトラン110mg 2回投与群ではワルファリン投与群と同等であった(**図1**)[4]．一方，大出血の発現率はダビガトラン110mg 2回投与群でワルファリン投与群より有意に少なく，ダビガトラン150mg 2回投与群でワルファリン投与群と同等であった(**図2**)[7]．また，出血性脳卒中の発症率は，いずれのダビガトラン投与群もワルファリン投与群より有意に少なかった(**図3**)[4]．

2010年10月，米国食品医薬品局(FDA)は150mgのダビガトランを承認し，続いてカナダでは110mgと

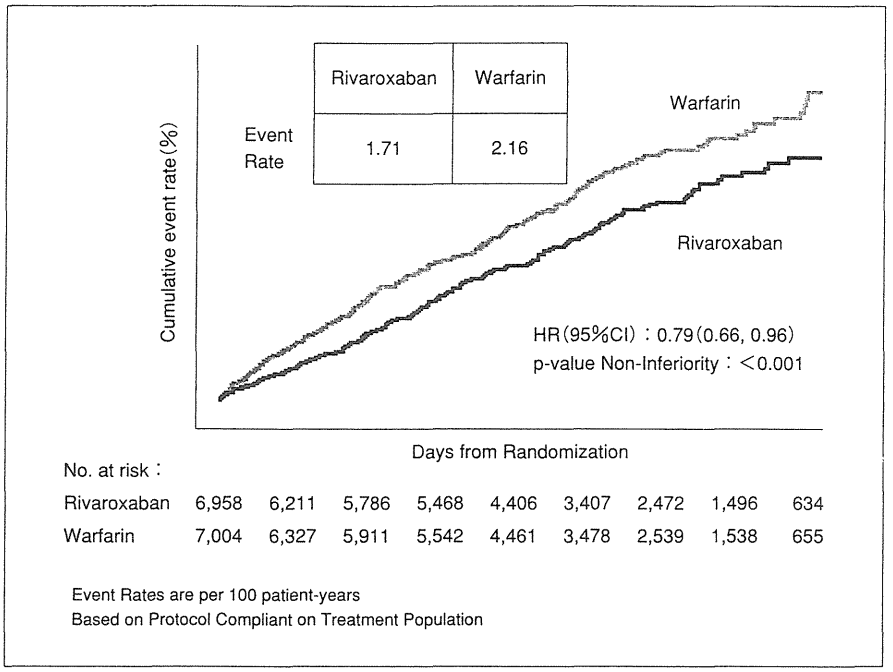

図 4
ROCKET AFの成績
－脳卒中または全身性塞栓症－
（文献6より改変引用）

150mg（1日2回投与）の両用量が承認され，日本でも2011年1月に異例の速さで両用量が承認され，3月から処方されるようになった．最近，欧州連合（EU）でも両用量が承認された．

その後，日本人のサブ解析も行われ，全体の成績と同様な有効性と安全性のプロフィールが示された[5]．すなわち，脳卒中・全身塞栓症の年間発症率は高用量ダビガトラン群1.11%，低用量ダビガトラン群1.54%，ワルファリン群1.71%であり，大出血は高用量ダビガトラン群3.32%，低用量ダビガトラン群2.87%，ワルファリン群3.57%であった[5]．

○ Xa因子阻害薬

複数のXa因子阻害薬が開発されたが，その中で心房細動患者においてワルファリンとの比較試験が最も先行していたのはリバロキサバンであった[2]．脳梗塞，TIA，非中枢神経系塞栓症の既往を有するか，心不全または左室駆出率35%以下，高血圧，75歳以上，糖尿病のうちの2つ以上を有する20歳以上の心房細動患者14,000例をリバロキサバン投与群（15mgまたは20mg，1回投与）かワルファリン投与群（INR 2～3，目標値2.5）に無作為割付して12～32カ月間追跡調査し，安全性の主要評価項目として重大な出血または臨床的に問題となる出血を調査し，有効性の主要評価項目として脳卒中および非中枢神経系塞栓症の発現を調査する二重盲検比較試験（Rivaroxaban Once-Daily Oral Direct Factor Xa Inhibition Compared with Vitamin K Antagonism for the Prevention of Stroke and Embolism Trial in Atrial Fibrillation；ROCKET-AF）が行われた[6]．On treatment解析において，脳卒中および非中枢性塞栓症の発症率はリバロキサバン投与群でワルファリン投与群より有意に少なく，ワルファリンに対するリバロキサバンの非劣性が示された（図4）[6]．大出血はリバロキサバン投与群で3.69%，ワルファリン投与群で3.45%であり，両群間に有意差はなかった（ハザード比1.04，95%信頼区間0.90～1.20，p＝0.576）．頭蓋内出血はリバロキサバン投与群（0.49%）でワルファリン投与群（0.74%）より有意に少なかった（ハザード比0.67，95%信頼区間0.47～0.94，p＝0.019）[6]．

日本では，ROCKET-AFと同様なプロトコールで1,280例をリバロキサバン投与群（10mgまたは15mg，

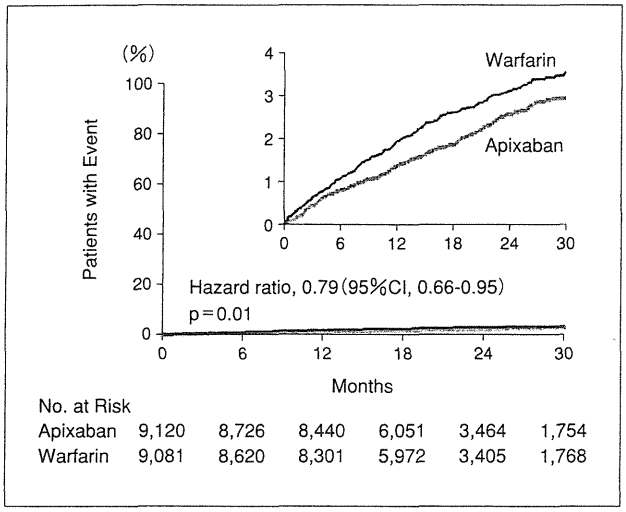

図5 ARISTOTLEの成績－脳卒中または全身塞栓症－
（文献8より改変引用）

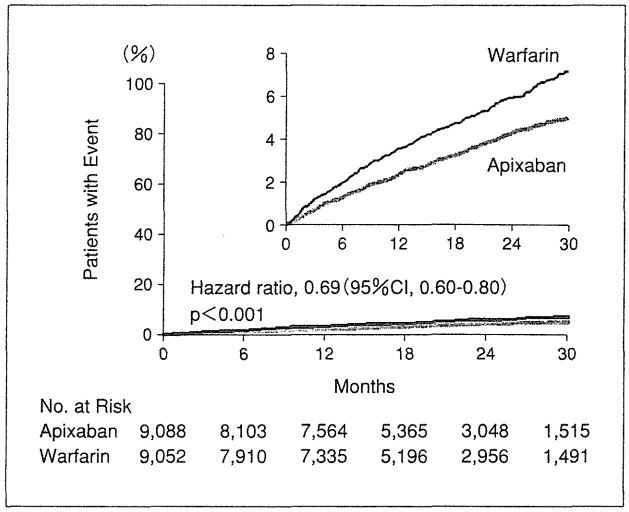

図6 ARISTOTLEの成績－大出血－
（文献8より改変引用）

1回投与）かワルファリン投与群（INRは70歳未満が2～3，70歳以上が1.6～2.6）に無作為割付し，同様な観察期間で同様な安全性と有効性の評価項目を調査する二重盲検比較試験（J-ROCKET AF）が行われた[7]．安全性の1次エンドポイントである大出血と臨床的に問題となる出血は両群間で差がなく（ハザード比1.11，95%信頼区間0.87～1.42），有効性の1次エンドポイントである脳卒中または全身塞栓症はリバロキサバン投与群（1.26%）でワルファリン投与群（2.61%）より少ない傾向を認めた（ハザード比0.49，95%信頼区間0.24～1.00，p＝0.050）[7]．

アピキサバンのワルファリンに対する非劣性を証明するためのARISTOTLE（Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation）試験が行われた[8]．本試験は，少なくとも1つの脳卒中の危険因子を有するNVAF患者18,206例において，アピキサバンのワルファリンに対する非劣性を検証する二重盲検試験であり，観察期間は12カ月～4年であったが，有効性と安全性の両方でアピキサバンはワルファリンを有意に上回るという成績が示された[8]．すなわち，脳卒中または全身塞栓症はアピキサバン投与群でワルファリン投与群より有意に少なく（**図5**），大出血もアピキサバン投与群でワルファリン投与群より有意に少なかった（**図6**）[8]．

エドキサバンは第一三共株式会社が開発した国産のXa因子阻害薬であり，$CHADS_2$スコア* 2点以上の高リスクの心房細動患者16,500例を対象としてエドキサバンの30mg（低用量）と60mg（高用量）のワルファリンに対する非劣性を検証する第3相臨床試験（Effective Anticoagulation with Factor Xa Next Generation in Atrial Fibrillation；ENGAGE AF）が，日本を含む46カ国が参加する国際共同研究として行われている[10]．本試験は中間観察期間が24カ月であり，2012年3月に終了予定である．

○ まとめ

これまでに報告された新規経口抗凝固薬の成績に

＊Congestive heart failure（うっ血性心不全），Hypertension（高血圧），Age≧75（75歳以上），Diabetes Mellitus（糖尿病），Stroke/TIA（脳卒中/一過性脳虚血発作）の頭文字をとって命名されたスコアで，前4つの項目には1点を，脳梗塞発症リスクの高いStroke/TIAの既往には2点を付与し，合算して算出する．合計点数が高いほど脳梗塞発症の危険性が高いと判断される（文献9より改変引用）．

はいくつかの共通点が認められる．すなわち，ワルファリンと比較して，脳卒中または全身塞栓症の予防効果は同等またはそれ以上であり，大出血は同等またはより少なく，頭蓋内出血(出血性脳卒中)は明らかに少ない点である[11]．一方，相違点としては，オープンラベルと二重盲検，1日1回投与と2回投与，危険因子数，ワルファリン投与群のコントロール状況の指標であるTTR(time in therapeutic range)，統計解析法と統計学的検出力があげられ，RE-LY，ROCKET AF，ARISTOTLEの成績からダビガトラン，リバロキサバン，アピキサバンの有効性と安全性を比較することは困難であり，現時点で優劣を論じるのは危険である[10]．2012年に結果が発表されるエドキサバンも含めて心房細動患者の抗凝固療法は新時代に突入したことは間違いないが，血液凝固モニターによる監視が可能な安心感，出血合併症発現時に使用可能な中和薬の存在，極めて低い薬価という観点からはワルファリンが優れており，今後もワルファリンは経口抗凝固薬の選択肢として生き残るであろう[11]．

文　献

1) Weitz JI, Bates SM : New anticoagulants. *J Thromb Haemost* 2005 ; **3** : 1843-1853
2) 内山真一郎：心房細動に対する抗血栓療法の有効性と安全性を探る　新しい抗凝固療法を展望する．*Vascular Med* 2007 ; **3** : 54-59
3) 内山真一郎：経口直接トロンビン阻害剤．*Pharm Med* 2009 ; **27** : 143-149
4) Connolly SJ, Ezekowitz MD, Yusuf S, et al : Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2009 ; **361** : 1139-1151
5) Hori M, Connolly SJ, Ezekowitz MD, et al : Efficacy and safety of dabigatran vs. warfarin in patients with atrial fibrillation--sub-analysis in Japanese population in RE-LY trial. *Circ J* 2011 ; **75** : 800-805
6) Patel MR, Mahaffey KW, Garg J, et al : Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. *N Engl J Med* 2011 ; **365** : 883-891
7) Hori M on behalf of the J-ROCKET AF Steering Committee and the J-ROCKET AF Investigators : J-ROCKET AF : the safety and efficacy of rivaroxaban for prevention of stroke in Japanese patients with nonvavular atrial fibrillation. International Congress of Thrombosis and Hemostasis 2011, Kyoto, 2011
8) Granger CB, Alexander JH, McMurray JJ, et al : Apixaban versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2011 ; **365** : 981-992
9) 小川　聡，相澤義房，新　博次，ほか：循環器病の診断と治療に関するガイドライン(2006－2007年度合同研究班報告)　心房細動治療(薬物)ガイドライン(2008年改訂版)．*Circ J* 2008 ; **72**(Suppl Ⅳ) : 1581-1638
10) Ruff CT, Giugliano RP, Antman EM, et al : Evaluation of the novel factor Xa inhibitor edoxaban compared with warfarin in patients with atrial fibrillation : design and rationale for the Effective aNticoaGulation with factor xA next GEneration in Atrial Fibrillation-Thrombolysis In Myocardial Infarction study 48(ENGAGE AF-TIMI 48). *Am Heart J* 2010 ; **160** : 635-641
11) Mega JL : A new era for anticoagulation in atrial fibrillation. *N Engl J Med* 2011 ; **365** : 1052-1054

Original Paper

Cerebrovascular Diseases

Cerebrovasc Dis 2012;34:385–392
DOI: 10.1159/000343659

Received: July 31, 2012
Accepted: September 19, 2012
Published online: November 29, 2012

Association of Serum Anti-Periodontal Pathogen Antibody with Ischemic Stroke

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Key Words
Periodontitis · Cerebral infarction · Atherosclerosis · Infectious disease · Atrial fibrillation

Abstract
Background: Periodontitis increases the risk of atherosclerotic cardiovascular disease and ischemic stroke. In this study, we evaluated whether serum antibody levels against individual periodontal pathogens are significantly associated with ischemic stroke subtypes and their risk factors. ***Methods:*** Patients with acute ischemic stroke (n = 132; 74 male and 58 female, 71.3 ± 10.7 years) and patients with no previous stroke (n = 77; 38 male and 39 female, 70.7 ± 9.5 years) were consecutively enrolled in this study. Stroke subtype was evaluated based on the Trial of Org 10172 in Acute Stroke Treatment classification. Serum was obtained from each patient after obtaining their consent to participate in the study. The levels of serum antibodies against *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg) and *Prevotella intermedia* (Pi) were evaluated by ELISA. Serum high-sensitivity C-reactive protein (hs-CRP) levels were measured by nephelometry. ***Results:*** Serum hs-CRP levels were significantly associated with acute ischemic stroke even after controlling for acute ischemic stroke, hypertension, diabetes mellitus and bulb/ internal carotid artery (ICA) atherosclerosis which were statistically selected (coefficient 0.245, 95% CI 0.142–0.347, $p < 0.0001$). The serum-antibody level of Pi was significantly higher in atherothrombotic-stroke patients than in patients with no previous stroke ($p = 0.0035$). Detectable serum anti-Pg antibody was significantly associated with atrial fibrillation (overall $\chi^2 = 35.5$, $R^2 = 0.18$, $n = 209$, $p < 0.0001$; anti-Pg antibody: OR 4.36, 95% CI 1.71–12.10, $p = 0.0017$), and detectable serum anti-Pi antibody was significantly associated with bulb/ICA atherosclerosis after controlling for the statistically selected associ-

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ated factors (overall $\chi^2 = 46.1$, $R^2 = 0.18$, n = 209, $p < 0.0001$; anti-Pg antibody: OR 16.58, 95% CI 3.96–78.93, $p < 0.0001$). The levels of serum anti-Pi antibody were significantly associated with atherothrombotic stroke with the statistically selected associated factors excluding bulb/ICA atherosclerosis (overall $\chi^2 = 77.0$, $R^2 = 0.44$, n = 129, $p < 0.0001$; anti-Pi antibody: OR 23.6, 95% CI 2.65–298.2, p = 0.008). However, when we included bulb/ICA atherosclerosis in this model, the levels of serum anti-Pi antibody were no longer significantly associated with atherothrombotic stroke (overall $\chi^2 = 98.0$, $R^2 = 0.56$, n = 129, $p < 0.0001$; anti-Pi antibody: p = 0.107). ***Conclusions:*** Our results suggest that anti-Pg antibody is associated with atrial fibrillation and that anti-Pi antibody is associated with carotid artery atherosclerosis. In addition, anti-Pi antibody may be associated with atherothrombotic stroke through its association with carotid artery atherosclerosis. Thus, periodontitis may lead to serious systemic diseases.

Introduction

Stroke is largely explained by several generally accepted risk factors; these do not, however, fully account for stroke epidemiology. Increasing evidence indicates that recent acute infection and various chronic infectious diseases are important risk factors for stroke. In a combined analysis of 2 prospective studies, periodontitis was found to increase the risk of stroke nearly 3-fold [1]. More recently, a larger case-control study confirmed the graded association between the severity of periodontitis and the risk of stroke [2]. Prospective case-control studies have identified patients who have infections caused by major periodontal pathogens as being at risk for future stroke [3, 4]. On the other hand, periodontal pathogens have been detected with other microbial antigens in carotid plaques. In addition, herpes simplex virus infection was recently reported to be associated with an increased risk of future atrial fibrillation [5]. Atrial fibrillation is also reported to be associated with inflammation [6]; however, its associations with other infectious diseases such as periodontitis are not well defined.

In this study, we evaluated whether serum antibody levels against individual periodontal pathogens are significantly associated with ischemic stroke subtypes and their risk factors. *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg) and *Prevotella intermedia* (Pi), three major periodontitis pathogens, were selected because of their reported associations with systemic diseases.

Methods

Subjects

A total of 132 consecutive patients with acute ischemic stroke were admitted to the Kagawa University Hospital within 24 h after stroke onset between January 1, 2005 and December 31, 2008. The control patients were consecutively enrolled when they were over 45 years old and they were subjected to brain magnetic resonance imaging (MRI) to detect any relevant non-stroke-related issues. They were excluded when they had any neurological deficit or had had a stroke previously (detected as signal loss in fluid-attenuated inversion recovery with MRI); 77 were ultimately used as control patients in the study. They attended our outpatient clinic and exhibited atypical symptoms. Informed consent was obtained from all patients. This study was approved by the investigational review board of the Kagawa University Hospital (H16–26).

Stroke specialists diagnosed ischemic stroke patients with cardioembolic, atherothrombotic or lacunar stroke, or any other type of stroke ('other') using echocardiography, brain computed tomography, MRI, magnetic resonance angiography and carotid ultrasonography. The final diagnosis of the stroke subtype was made before discharge, based on the Trial of Org 10172 in Acute Stroke Treatment classification [7].

Detailed data were collected from all patients by physicians, including baseline characteristics (age, sex, blood pressure and drinking and smoking habits) and vascular risk factors (hypertension, diabetes mellitus and dyslipidemia).

Hypertension, diabetes mellitus and dyslipidemia were diagnosed by physicians. Patients were designated as hypertensive if they were taking antihypertensive agents and had a systolic blood pressure ≥140 mm Hg and/or a diastolic blood pressure ≥90 mm Hg. Patients were diagnosed with diabetes mellitus if they were treated with oral hypoglycemic agents or insulin and/or their serum fasting blood glucose level was ≥7 mmol/l. Patients were diagnosed with dyslipidemia if they were taking antidyslipidemia medication or presented the following levels: serum LDL-cholesterol ≥3.62 mmol/l, triglycerides ≥1.69 mmol/l and/or HDL-cholesterol <1.03 mmol/l.

In this study, a patient was classified as 'drinker' if they had at least one alcoholic drink per day. Occasional drinkers and nondrinkers were both classified as 'nondrinkers'. A patient was classified as a 'smoker' if they smoked at the time of the study or had quit smoking less than 1 year previously. Those patients who had quit smoking more than 1 year previously and those who had never smoked were classified as 'nonsmokers'. These categories were assigned by a physician who interviewed the patient and/or the patient's family.

Carotid Ultrasonography

Carotid ultrasound examinations were performed by one trained physician (H.O.) with a 5- to 10-MHz annular array transducer connected to an ultrasound imaging system (LOGIQ 500; General Electric Yokogawa Medical Systems, Tokyo, Japan) with a monitor that displayed the electrocardiogram, as previously reported [8, 9]. Bulb/internal carotid artery (ICA) atherosclerosis was diagnosed when a plaque [intima-media thickness (IMT) ≥1.1 mm] was detected [10].

Table 1. Baseline characteristics

	No previous stroke (n = 77)	Ischemic stroke (n = 132)	p value
Mean age ± SD, years	70.7 ± 9.5	71.3 ± 10.7	0.6782
Female sex, n (%)	39 (50.7)	58 (43.9)	0.3895
Hypertension, n (%)	20 (26.0)	83 (62.9)	<0.0001
Diabetes mellitus, n (%)	34 (44.2)	39 (29.6)	0.0362
Dyslipidemia, n (%)	28 (36.4)	41 (31.1)	0.4491
Drinking, n (%)	16 (20.8)	40 (30.3)	0.1478
Smoking, n (%)	18 (23.4)	37 (28.0)	0.5169
Atrial fibrillation, n (%)	8 (10.4)	31 (23.5)	0.0262
Bulb/ICA atherosclerosis, n (%)	15 (19.5)	49 (37.1)	0.0082
Subtypes of ischemic stroke			
Lacunar stroke, n (%)	0	42 (31.8)	N.D.
Atherothrombotic stroke, n (%)	0	52 (39.4)	N.D.
Cardioembolic stroke, n (%)	0	38 (28.8)	N.D.
hs-CRP, log, ng/ml	2.75 ± 0.69	3.23 ± 0.67	<0.0001

N.D. = Not determined.

Quantification of Serum Anti-Periodontal Pathogen Antibodies

Serum samples were obtained from patients with acute ischemic stroke upon hospital admission and from those without previous stroke at an outpatient clinic. The samples were kept at −78°C until testing. The level of serum antibody against Aa, Pg or Pi was quantified using the enzyme-linked immunosorbent assay (ELISA), as reported previously [11]. The strains of periodontitis pathogens were ATCC33384, ATCC33277 and ATCC25611 for Aa, Pg and Pi, respectively. Each suspension was incubated at 4°C overnight in 96-well microtiter plates for coating. The reference IgG was purified from serum with a high IgG level to the periodontal pathogens of interest. The diluted reference IgG and vehicle (phosphate-buffered saline) were used to construct standard curves. The patient serum or the reference IgG was applied in duplicate to the wells, and the plates were incubated for 1 h. After washing, the wells were incubated for 1 h with anti-human IgG labelled with peroxidase (DakoCytomation, Carpinteria, Calif., USA). The plates were washed and incubated with the peroxidase substrate (3,3′,5,5′-tetramethylbenzidine) for 30 min. The optical density was read at a wavelength of 450 nm and a sub-wavelength of 650 nm on a plate reader. The serum IgG antibody levels of each patient were calculated using the IgG reference curves and were expressed in relative arbitrary ELISA units. The coefficients of variation were 7.63, 7.19, and 8.12 (original values) and 1.34, 1.21, and 1.42% (logarithmic values) for anti-Aa antibody, anti-Pg antibody and anti-Pi antibody levels, respectively (n = 6). We blinded the physicians who diagnosed the ischemic stroke subtypes to the serum anti-periodontal-pathogen antibody values.

Quantification of Serum High-Sensitivity C-Reactive Protein

Serum high-sensitivity C-reactive protein (hs-CRP) levels were measured by nephelometry (NA Latex CRP kit; Dade Behring) in SRL, Inc. (Tokyo, Japan). When the hs-CRP value was below the limit of detection (50 ng/ml), it was recorded as 50 ng/ml.

Statistical Analysis

The levels of serum anti-periodontal-pathogen antibodies and serum hs-CRP were analyzed in logarithmic form because these values exhibit a Gaussian distribution. The data were expressed as the means ± standard deviations (SD) for the continuous variables and as frequencies and percentages for discrete variables. Univariate analyses were performed to evaluate the differences between the groups regarding the baseline characteristics, risk factors and levels of serum hs-CRP and serum anti-periodontal-pathogen antibodies. The groups were compared using a one-way analysis of variance (ANOVA; for continuous variables) or the Fisher exact test (for discrete variables). As there are many independent variables, two-step regression strategies were pursued. First, a forward stepwise regression was used with no forced independent variables unless otherwise mentioned and p values of 0.25 to enter and remove. The linear regression model or the logistic regression model reported in the next section with selected factors that were determined from the forward stepwise regression.

Statistical analysis was performed using JMP software version 9.0 for Macintosh. All analyses were two-tailed and a value of $p < 0.05$ was considered statistically significant.

Results

We evaluated 132 acute ischemic-stroke patients and 77 patients with no previous stroke. Baseline characteristics and the levels of serum hs-CRP are shown in table 1. In the patients with the acute ischemic stroke, prevalence of hypertension, atrial fibrillation and bulb/ICA atherosclerosis was significantly higher than that of the patients with no previous stroke. In contrast, the prevalence of

Table 2. Serum anti-periodontal-pathogen antibody levels

	Aa, logU/ml	Pg, logU/ml	Pi, logU/ml
Age (years) (n = 209)	$R^2 = 0.0058$	$R^2 = 0.0004$	$R^2 = 0.0002$
Gender			
Female (n = 97)	1.68 ± 0.47	1.82 ± 0.49	2.42 ± 0.30
Male (n = 112)	1.66 ± 0.50	1.96 ± 0.44*	2.52 ± 0.28*
Hypertension			
No (n = 106)	1.71 ± 0.51	1.91 ± 0.48	2.44 ± 0.29
Yes (n = 103)	1.63 ± 0.47	1.88 ± 0.45	2.50 ± 0.30
Diabetes mellitus			
No (n = 136)	1.67 ± 0.46	1.87 ± 0.48	2.45 ± 0.32
Yes (n = 73)	1.67 ± 0.54	1.94 ± 0.45	2.51 ± 0.24
Dyslipidemia			
No (n = 140)	1.61 ± 0.45	1.88 ± 0.46	2.41 ± 0.28
Yes (n = 69)	1.79 ± 0.53*	1.93 ± 0.48	2.61 ± 0.27***
Drinking			
No (n = 153)	1.66 ± 0.47	1.84 ± 0.49	2.47 ± 0.29
Yes (n = 56)	1.69 ± 0.53	2.03 ± 0.39**	2.49 ± 0.29
Smoking			
No (n = 154)	1.70 ± 0.47	1.91 ± 0.46	2.46 ± 0.29
Yes (n = 55)	1.60 ± 0.53	1.86 ± 0.50	2.50 ± 0.30
Atrial fibrillation			
No (n = 170)	1.65 ± 0.48	1.83 ± 0.46	2.45 ± 0.29
Yes (n = 39)	1.74 ± 0.51	2.15 ± 0.41***	2.58 ± 0.28**
Bulb/ICA atherosclerosis			
No (n = 145)	1.64 ± 0.47	1.88 ± 0.46	2.41 ± 0.29
Yes (n = 64)	1.74 ± 0.53	1.93 ± 0.49	2.61 ± 0.25***
Stroke			
No previous (n = 77)	1.74 ± 0.54	1.86 ± 0.51	2.44 ± 0.28
Acute ischemic (n = 132)	1.63 ± 0.45	1.91 ± 0.44	2.49 ± 0.30
hs-CRP, log, ng/ml (n = 209)	$R^2 = 0.0085$	$R^2 = 0.0002$	$R^2 = 0.0008$

All values are presented as the mean ± SD in logarithmic form. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.005$.

diabetes mellitus was higher in the patients with no previous stroke. In the acute ischemic stroke group, serum hs-CRP levels were significantly elevated compared to the levels in patients with no previous stroke ($p < 0.0001$). With a forward stepwise regression, acute ischemic stroke, hypertension, diabetes mellitus and bulb/ICA atherosclerosis were selected as factors that associated with serum hs-CRP levels. Serum hs-CRP levels were significantly associated with acute ischemic stroke even after controlling for those statistically selected associated factors (coefficient 0.245, 95% CI 0.142–0.347, $p < 0.0001$).

The association between the baseline characteristics and the levels of serum anti-periodontal-pathogen antibodies were evaluated (table 2). The levels of serum anti-Aa antibody were higher in patients with dyslipidemia than in patients without. The levels of serum anti-Pg antibody were higher in male patients and patients with drinking habit and atrial fibrillation than in patients without these conditions. The levels of serum anti-Pi antibody were higher in male patients and patients with dyslipidemia, atrial fibrillation and bulb/ICA atherosclerosis than in patients without these conditions. In this study, no significant differences were identified between the groups in terms of serum anti-periodontal-pathogen antibodies and smoking habits.

To assess the association of the levels of serum anti-periodontal-pathogen antibodies with stroke risk factors, the association of the levels of serum anti-periodontal-pathogen antibodies with atrial fibrillation and the bulb/ICA atherosclerosis was evaluated. There were 39 patients (18.7%) with atrial fibrillation in total. The levels of serum antibodies of Pg and Pi, but not of Aa, were significantly

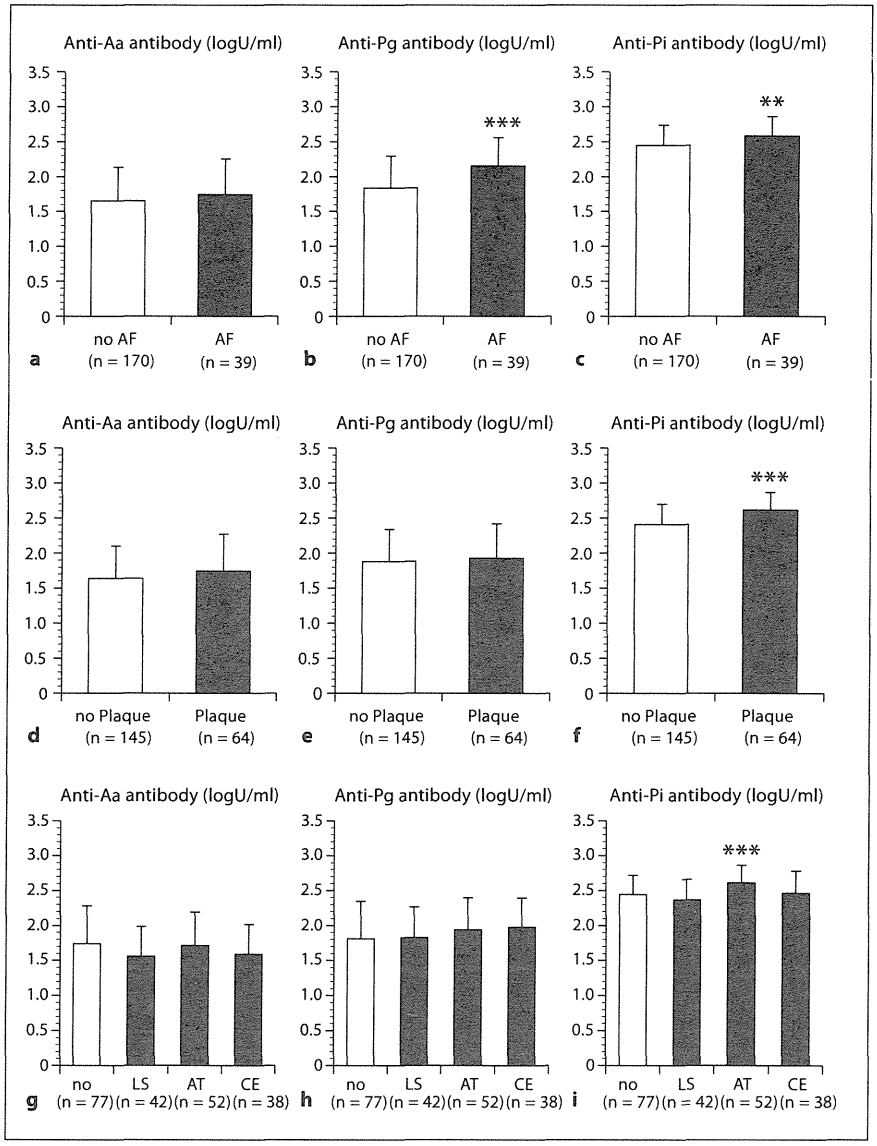

Fig. 1. Differences in the levels of serum anti-periodontal-pathogen antibodies between with/without atrial fibrillation, with/without bulb/ICA atherosclerosis and the subtypes of ischemic stroke. Levels of serum antibody against Aa (**a**), Pg (**b**) and Pi (**c**) in patients with atrial fibrillation (AF) versus the patients without it (no AF). Levels of serum antibody against Aa (**d**), Pg (**e**) and Pi (**f**) in patients with bulb/ICA atherosclerosis (plaque) versus the patients without it (no plaque). Levels of serum antibody against Aa (**g**), Pg (**h**) and Pi (**i**) in the patients with lacunar stroke (LS), atherothrombotic stroke (AT) or cardioembolic stroke (CE) were compared to those in patients with no previous stroke (no). ** $p < 0.05$ and *** $p < 0.005$ compared to the patients without these conditions.

higher in the patients with atrial fibrillation compared to that in patients without it ($p < 0.0001$ and $p = 0.0093$; respectively, fig. 1a–c; table 2). With a forward stepwise regression forced to include the levels of serum antibodies against Aa, Pg and Pi, age and hypertension were selected as factors that associated with atrial fibrillation. The level of serum anti-Pg antibody was significantly associated with atrial fibrillation even after controlling for those statistically selected associated factors (overall $\chi^2 = 35.5$, $R^2 = 0.18$, $n = 209$, $p < 0.0001$. Anti-Pg antibody: OR 4.36, 95% CI 1.71–12.10, $p = 0.0017$; fig. 2a), but not with the levels of serum antibodies against Aa and Pi.

There were 64 patients (30.6%) with bulb/ICA atherosclerosis in total. The levels of serum anti-Pi antibody were significantly higher in the patients with bulb/ICA atherosclerosis than that in patients without it ($p < 0.0001$), but not when compared to the levels of serum antibodies against Aa and Pg (fig. 1d–f; table 2). With a forward stepwise regression forced to include the levels of serum antibodies against Aa, Pg and Pi, sex, hypertension, diabetes mellitus, dyslipidemia, drinking and serum hs-CRP levels were selected as factors that associated with bulb/ICA atherosclerosis. Bulb/ICA atherosclerosis was significantly associated with the level of