Atrial fibrillation (AF), one of the most common arrhythmias, has grown to be an important medical problem in societies with an increasing number of aged people, because AF is strongly associated with the occurrence of severe thromboembolism. Although the processes underlying AF-associated thrombosis have long been believed to be mainly dependent upon the decreased blood flow in the left atrium induced by AF, revisiting the well known Virchow's triad from the basic approach has disclosed that this is too simplistic. Here, the role of 3 important components, abnormalities in the blood flow, blood coagulability, and the endocardial function of the atria, in thrombus formation in the fibrillating atria are discussed. Unraveling the molecular basis of thrombus formation in the atrium could open a new era of a wide variety of management of stroke prevention for AF patients.

Key Words: Atrial fibrillation; Endocardium; Thrombosis

Atrial fibrillation (AF), the most common type of arrhythmia in adults, is associated with a 5-fold increase in the incidence of ischemic stroke. Stroke in AF patients is believed to be mostly cardioembolic, caused by embolism of left atrial (LA) thrombi and is an important cause of hospitalizations and cardiovascular deaths, which simultaneously increases the public health cost of managing AF. A recent report revealed that the medical cost of AF was approximately 1% of the total and mainly resulted from the treatment of cerebrovascular events associated with AF. Also, recent clinical trials, including the AFFIRM study, could not demonstrate any differences between rhythm and rate control strategies for AF on patient outcomes, implying that the prevention of stroke by anticoagulation therapy is mandatory and the first step for decreasing the cardiovascular events in these patients. Therefore, total management of AF, complications rather than AF per se, is required.

Although it has been quite naturally believed that AF induces thrombus formation in the LA most easily by decreasing the blood flow in the atrium, this is not the case, as many previous studies have demonstrated that not all AF patients are at a high risk of stroke. The presence of LA thrombus in AF patients is only one of the results of AF, and many unidentified processes would be operating under thrombus formation in association with AF before the unfortunate event. Actually, it has not been observed in clinical patients how thrombus gradually forms in the fibrillating atria and also how the thrombus separates from the endocardium, leading to systemic thromboembolism. Therefore, suggesting that thromboembolism in AF patients is caused solely by decreased blood flow may be too simplistic. Rather, it should be noted that many processes exist under the thrombus formation in fibrillating atria and that they have remained unclear for a long time.

Over 150 years ago, Virchow postulated that 3 factors predispose to thrombus formation in venous thrombosis: abnormalities in blood flow, blood constituents and the vessel wall. His concept has been believed to be true for more than 100 years and also applied to arterial thrombosis. Thrombus formation in the fibrillating LA has more similarities to thrombosis in the vein than in the artery, because it occurs when blood flow is restricted. Therefore, it would be appropriate to consider the processes of thrombus formation in the atrium in view of Virchow’s triad. In the fibrillating atria, the triad corresponds to a decrease in blood flow, abnormalities in coagulation, and atrial endocardial dysfunction. Although these components are invariably and mutually associated with each other, the basic information regarding each component would bring insights into the pathophysiology of AF-associated thrombosis, as well as helping to identify potential therapeutic strategies.

Decreased Blood Flow in the Fibrillating Atrium

Decreased blood flow in the fibrillating LA is evident in AF patients and has been believed to play the major role in thrombus formation. Because blood is a fluid with particles (red blood cells, white blood cells, platelets, and etc) in suspension, its flow conditions are essential for determining the blood status.

Quantification of flow properties in the LA can be made using transesophageal echocardiography. Many previous studies have demonstrated that the blood flow in the LA appendage is significantly reduced in AF patients with thrombus or history of stroke, as compared with those not having such complications. It should be noted, however, that most of these studies are retrospective, evaluating AF patients with varying risk factors including age, hypertension, diabetes mellitus and congestive heart failure. Therefore, many problems remain to be solved. (1) Are all of AF patients with reduced blood flow in the LA at a high risk of developing stroke? (2) What are the relationships between
the stroke risk factors and the blood flow in the LA? (3) Can blood flow (cm/s) predict the occurrence of stroke as a continuous variable? To answer these questions, prospective long-term studies with standardized therapeutic regimens are required.

In considering the role of decreased blood flow in the LA, a report from the SPAF III trial presents an interesting and useful result.

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Increased Blood Coagulability in AF

For thrombus formation in the LA, Virchow’s triad suggested that there must be an increased level of the relevant blood constituents that create a hypercoagulable state. Although many of the candidate constituents have been proved to be increased in AF patients, and most of them are related to the coagulation and fibrinolytic cascade systems, the following problems are still undetermined. (1) What is the role of each increased constituent representing the hypercoagulable state for thrombus formation? (2) Are the increased levels the cause or result? (3) What are the relationships between the increased levels and the characteristics of the patient (eg, presence of AF, age, hypertension)?

In arterial thrombosis, the physiologic responses to vascular damage culminate in the rapid adhesion of platelets at the site of injury, which activate themselves to promote the coagulation cascade. Localization of several reactions, which is achieved by the binding of circulating coagulation proteins to the damaged vascular wall and/or platelets, is mandatory for providing rapid activation of coagulation, and thus thrombus formation. However, differently from these pathophysiologic processes in vascular thrombi, little is known regarding the initiation process of thrombus formation in the fibrillating LA. Because it is formed under conditions of slow blood flow, the activation of the coagulation cascade, rather than platelet activation, has been believed to be important though this concept has not been clearly proved.

Traditionally, the coagulation cascade is divided into 2 pathways: the extrinsic or contact system, and the intrinsic system. The intrinsic formation of factor Xa is a 5-component complex system and requires factor X, factor IXa, factor VIII, a reaction surface, and calcium ions simultaneously. Therefore, the extrinsic pathway is now supposed to be the dominant mechanism of initiating hemostasis. However, the involvement of tissue factor, which is an injury-based lipoprotein and functions as a cell-surface receptor for factor VII in vascular thrombus formation, has never been shown to play a significant role in thrombus formation in the fibrillating atrium. The initiating processes in the activation of the coagulation cascade in the atrium remain to be clarified.

Ultimately, via activation of the coagulation cascade, factor Xa is activated to factor Xa and leads to formation of thrombin, which in turn cleaves fibrinogen to fibrin. Clinically, the reaction of these coagulation factors can be evaluated by measuring several molecules in venous blood drawn from patients (Table 1). Although many abnormal indices have been noted in AF patients, the interpretation of the relationship between these findings and the likelihood of thrombus formation is too difficult, because most of the markers are only indices of coagulation activation, not of coagulation initiation. If a patient with AF has LA thrombi, all of the markers for coagulation and fibrinolytic processes would be released from the surface of thrombi and should be increased. Therefore, abnormalities in these markers can identify AF patients at high risk, but do not indicate that abnormalities in blood constituents play a major role in thrombus formation in the fibrillating atrium. The SPAF III trial could not demonstrate any relationships between the serum level of markers in F1+2 and the subsequent occurrence of stroke in high-risk AF patients.

There is considerable controversy among investigators whether AF itself activates the coagulation system (Table 2). The results from the Framingham study, however, revealed the difficulties in discussing this problem. The Framingham study has shown that AF patients have significantly increased levels of fibrinogen, von-Willebrand factor and t-PA compared with non-AF subjects, but at the same time demonstrated that these differences resulted from differences in the patients’ backgrounds (ie, age, hypertension, diabetes mellitus, and cardiovascular diseases), and not from the presence of AF. AF may be one of the outcomes of the many clinical risk factors for atherosclerosis which could activate the coagulation cascade in not only the arterial atheroma, but also in the atrium. However,

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**Table 1   Markers for Coagulation and Fibrinolytic Cascades**

<table>
<thead>
<tr>
<th>Category</th>
<th>Markers</th>
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<tbody>
<tr>
<td>(A) Activation of coagulation</td>
<td>1. Thrombin generation: TAT, F1+2</td>
</tr>
<tr>
<td></td>
<td>2. Fibrin generation: SFMC, FPA</td>
</tr>
<tr>
<td>(B) Activation of fibrinolysis</td>
<td>1. Plasmin degradation: PIC</td>
</tr>
<tr>
<td></td>
<td>2. Fibrin degradation: D-dimer, FDP</td>
</tr>
<tr>
<td>(C) Activation of platelets</td>
<td>1. r-PA</td>
</tr>
<tr>
<td>(D) Markers for endothelial damage</td>
<td>1. t-PA, PAI-1, TFP</td>
</tr>
</tbody>
</table>

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TAT, thrombin-antithrombin complex; F1+2, prothrombin fragments 1+2; SFMC, soluble fibrin monomer complex; FPA, fibrinopeptide A; PIC, plasmin-2-plasmin complex; FDP, fibrin degradation product; t-PA, tissue-plasminogen activator; PAI-1, plasminogen activator inhibitor-1; fTG, D-thromboglobulin; PF4, platelet factor 4; TM, thrombomodulin; vWF, von Willebrand factor; TFP, tissue factor pathway inhibitor.
AF in itself could directly alter blood viscosity\textsuperscript{26} because secretion of atrial natriuretic peptide induced by the occurrence of AF could lead to dehydration, which increases blood viscosity and also the plasma concentration of procoagulants.

Endocardial Dysfunction of the Atria in AF

Blood coagulation is a serial reaction of several coagulation factors, which interact with each other mainly on the membrane of activated platelets and other stimulated cells\textsuperscript{27} Because of the low concentration of these factors in plasma and the abundance of their inhibitors, the interaction of procoagulants and their activation can proceed only slowly in the fluid phase of blood\textsuperscript{17,27} Therefore, AF-associated thrombus formation requires first a localized surface of coagulation reactions, one candidate of which would be the atrial endocardium. Thus, abnormalities in endocardial function in the atria could offer an initiating step for coagulation\textsuperscript{28}

The normal endothelium of the vasculature has anticoagulation and antiplatelet functions\textsuperscript{27} Endothelial cells, to prevent blood clotting on the surface of the vasculature, express abundant anticoagulant molecules, including tissue factor pathway inhibitor (TFPI), anti-thrombin III, and thrombomodulin (TM), and also antiplatelet molecules such as prostacyclin and nitric oxide\textsuperscript{27} Both TM and TFPI play a pivotal role in maintaining the normal coagulation balance\textsuperscript{29,30} Endothelial TM forms a complex with thrombin and thereby changes its substrate specificity as an intrinsic thrombin inhibitor\textsuperscript{29} Thrombin bound to TM cannot convert fibrinogen to fibrin or activate protein C, a major anticoagulant protein. TFPI is a direct inhibitor of factor Xa activity and also a factor-X-dependent inhibitor of TF and factor VII/VIIa, thus being the most important physiologic inhibitor of TF-dependent coagulation\textsuperscript{30} Apart from these important molecules, it would be also important that normal endothelial cells do not express procoagulant molecules, such as tissue factor, plasminogen-activator inhibitor-1 (PAI-1), adhesion molecules, or chemokines\textsuperscript{27}

Until recently, little information has been available regarding the antithrombotic function of normal atrial endocardium. Kamiyama has for the first time reported that AF per se induces atrial endocardial dysfunction, an induction of adhesion molecules, in the rapidly-paced atria of rabbits\textsuperscript{31} Since then, many investigators have begun to pay attention to the function of the atrial endocardial cells, a barrier between the blood and atrial cardiomyocytes\textsuperscript{32–37} Cai et al demonstrated that AF decreased nitric oxide synthase expression and increased PAI-1 expression in the

Table 2  Reports of Alterations in Hemostatic Markers

<table>
<thead>
<tr>
<th>Coagulation</th>
<th>Platelet</th>
<th>Fibrinolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPA</td>
<td>TAT</td>
<td>F1+2</td>
</tr>
<tr>
<td>Stroke 1990; 21: 47</td>
<td></td>
<td></td>
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<td>Jpn Circ J 1994; 58: 821</td>
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<td></td>
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<td>Stroke 1995; 26: 1365</td>
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<tr>
<td>Am J Cardiol 1996; 77: 528</td>
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<tr>
<td>Thromb Haemost 1996; 75: 219</td>
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<tr>
<td>Am J Cardiol 1997; 79: 1131</td>
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<tr>
<td>CMAJ 1997; 157: 673</td>
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<tr>
<td>Am Heart J 1998; 136: 956</td>
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<td>Int J Cardiol 1998; 66: 153</td>
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<tr>
<td>Thromb Haemost 1999; 82: 100</td>
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<tr>
<td>Platelets 2003; 14: 407</td>
<td></td>
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</tr>
</tbody>
</table>

Fbg, fibrinogen. Other abbreviations see in Table 1.
Thromboembolism in AF

atal endocardium of pigs. My group have also shown that AF decreases the mRNA and protein expressions of TFPI and TM in rats. Moreover, it has been reported that hypertension, an important predisposing factor for AF, downregulates TM expression in the atrial endocardium. Several investigators have shown evidence that endocardial function is also impaired in human AF. Fukuchi et al reported that von Willebrand factor is present on the surface of the atrial endocardium in AF patients. Other investigators have demonstrated the expression of tissue factor in specimens from the atrium of AF patients undergoing valvular surgery. Taken together, it seems reasonable to speculate that the antithrombotic functions of the atrial endocardium are damaged by AF itself.

The processes underlying this impairment of the antithrombotic function of the atrial endocardium will be the future target of investigation. Recently, a mechanism of this endocardial dysfunction has been proposed: oxidative stress is produced in the LA by AF. Dudley et al have demonstrated that O2– production was increased in the pig LA by AF, primarily via increased activity of NADPH and xanthine oxidase. This observation suggests an analogy between AF and atherosclerosis in terms of linkage with local activation of the renin-angiotensin system, because angiotensin II is a potent stimulator of NAD(P)H oxidase. Moreover, these considerations are consistent with a recent report that atrial stretch, which increases oxidase production via the renin-angiotensin system, impairs the antithrombotic function of the endocardium, as shown by TM downregulation.

Therapeutic Considerations

From the era of the too simple consideration that decreased blood flow was the key to thrombus formation in AF, we have moved to a new era of the developed concept that the 3 important factors together contribute to thrombus formation in the fibrillating LA (Fig 1). Progress in our understanding of this concept should, hopefully, extend therapeutic approach to preventing thromboembolism in AF.

Improvement in blood flow alone is not a reliable method at present, because recent mega-trials have shown that pharmacological rhythm control was not associated with a significant decrease in stroke incidence. In contrast, improvement in the hypercoagulable state by warfarin therapy has been convincingly effective. Thrombin or Xa inhibitors that are now under clinical investigation are also very promising. In addition, improvement in the atrial endocardial function (ie, controlling the endocardial milieu) offers a hopeful strategy. For example, the LIFE study has demonstrated that the incidence of stroke in AF patients was significantly more decreased by losartan than by atenolol. Moreover, in the PROGRESS study, perindopril decreased stroke in AF patients with a history of cerebrovascular events. Interestingly, these preventive effects were independent of blood pressure and oral anticoagulation therapies, suggesting that an approach other than anticoagulation therapy is actually available.

Conclusions

Thromboembolism is one of the most important medical problems in the management of AF. Although anticoagulation therapy by warfarin has been the only solution for a long time, there is now ample evidence that other promising approaches will be available in the near future. This progress depends on revisiting Virchow’s triad. Unraveling the molecular basis of the 3 main components and their mutual interaction is expected to improve the risk stratification and management of AF patients.