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Full Length Article

Comparison of chronological changes in blood characteristics in the atrium and peripheral vessels after the development of non-valvular atrial fibrillation☆

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ABSTRACT

Introduction: Changes in blood characteristics in the atrium and peripheral vessels in patients with non-valvular atrial fibrillation (NVAF) are unclear. We investigated chronological changes in blood characteristics in the atrium and peripheral vessels in a dog model of NVAF by using a total thrombus-formation analysis system (T-TAS).

Materials and methods: In NVAF model dogs (n = 8, 390 bpm rapid atrial pacing), atrial and peripheral blood samples were collected. Using this blood, T-TAS was performed before and 1, 2, and 3 weeks after the onset of rapid atrial pacing.

Results: Occlusion time (OT: time to +80 and +60 kPa in the AR and PL chips, respectively) and area under the flow pressure curve (AUC) were measured using the AR chip (for mixed white thrombus analysis) and PL chip (for platelet thrombus analysis). OT of the AR chip showed shortening as early as 1 week after NVAF onset, which continued for 3 weeks. OT of the PL chip showed significant shortening in atrium blood only 3 weeks after NVAF onset. By contrast, peripheral blood showed no significant changes in OT or AUC with both AR and PL chips.

Conclusions: In our dog model of NVAF, thrombus formation accelerated in the atrium as early as 1 week after NVAF onset and continued for 3 weeks, but no significant changes were found in peripheral blood. We conclude that antithrombotic therapy should be started early after NVAF onset even if no changes in coagulation activity are observed in peripheral blood.

1. Introduction

Thromboembolism is a condition that involves blockage of blood flow by a thrombus generated in the heart and vessels [1]. In human medicine, non-valvular atrial fibrillation (NVAF) is the most common cause (45%) of cardiogenic cerebral embolism [2] and significant attention has focused on it as the causative factor of thromboembolism [3–5]. A low 1-year survival rate (approximately 50%) was reported in patients with cerebral infarction caused by cardiac disease-derived thrombus [6]. These findings warrant the development of a method for

Abbreviations: AHA/ACC, American Heart Association/American College of Cardiology; APTT, active partial thromboplastin time; AR chip, chip for mixed white thrombus analysis; ATIII, antithrombin III; AUC, area under the flow pressure curve; DAP, diastolic arterial blood pressure; ESC, European Society of Cardiology; Fibn, fibrinogen; MAP, mean arterial blood pressure; NVAF, non-valvular atrial fibrillation; OT, occlusion time; PFA-100, platelet function analyzer-100; PL chip, chip for platelet thrombus analysis; PT, prothrombin time; RAP, rapid atrial blood pacing; ROTEM, rotational thromboelastometry; SAP, systolic arterial blood pressure; SEC, spontaneous echo contrast; T_{10}, time to +10 kPa; T-TAS, total thrombus-formation analysis system

☆ Data statement

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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the early detection of thrombus or a tendency to develop thrombus.

The risk of developing NVAF-associated cerebral infarction can be evaluated using $CHADS_2$ (C: congestive heart failure, H: hypertension, A: age of ≥75 years, D: diabetes mellitus, S_2: stroke/TIA) or $CHADS_2$-VASc (V: vascular disease, A: age of 65–74 years, Sc: sex category) scores of the American Heart Association/American College of Cardiology (ACC/AHA) and European Society of Cardiology (ESC). Although not all high-risk patients develop thromboembolism, anticoagulant therapy has been provided for patients depending on their risk score [7–9]. Thrombus formation was visualized on transesophageal echocardiography [10–14]. However, this examination was not considered to be applicable to all patients because of its invasiveness. Therefore, the development of any alternative examination index other than clinical findings was considered essential for patients who require anticoagulant therapy.

In patients with NVAF, molecular markers of the coagulation system, including fibrinogen (Fibn) and factor VIII, were reported to increase in peripheral blood after thrombus formation [3,15–18]. Thrombus formation accelerated in the atrium in patients with NVAF. However, changes in blood characteristics in the atrium in such patients have yet to be evaluated. Furthermore, the time of occurrence of changes in blood characteristics in the atrium and peripheral vessels such as those in the extremities after the development of NVAF has not been demonstrated yet.

Recently, a medical apparatus, the total thrombus-formation analysis system (T-TAS; Fujimori Kogyou Co., Ltd., Japan) was developed for measurement of the functions of platelets and coagulation factors in conditions resembling blood flow in the living body [19–21]. Thanks to this system, thrombus formation in blood flow can now be evaluated using numerical and visual analyses. T-TAS has also been reported to be clinically applicable in dogs [22]. In the present study, an experimentally developed dog model of NVAF was used and chronological changes in blood characteristics were tested using the T-TAS to determine the risk of thrombus formation. In addition, conventional blood coagulation tests such as of prothrombin time (PT), active partial thromboplastin time (APTT), and fibrinogen (Fibn) level were performed.

2. Materials and methods

This study was conducted in accordance with the Ethical Code of Animal Experiments of Tokyo University of Agriculture and Technology (approval No. 27–39).

2.1. Test animals

Four male and four female healthy beagles were included in the study. The animals were 1 to 5 years old and had a body weight of 9.0 to 12.5 kg at the start of the study.

2.2. Methods for creating the NVAF model

Under general anesthesia, a pacing lead was implanted into the animals in accordance with the methods of Fukushima [23] and Gaspo et al. [24]. For stimulation of the atrium by rapid atrial pacing (RAP), artificial tachycardia was introduced and maintained in the dog, and hemodynamics resembling the spontaneously developed NVAF was obtained [23,24]. Briefly, the animals were preanesthetized with 30 μg/kg atropine sulfate (SC injection, atropine sulfate; Mitsubishi Tanabe Pharma Co., Osaka, Japan), 0.2 mg/kg butorphanol tartrate (IV injection, Betorufaru; Meiji Seika Pharma Co., Ltd., Tokyo, Japan), and 0.2 mg/kg midazolam (IV injection, Dormicum; Astellas Pharma, Tokyo, Japan). For the induction and maintenance of anesthesia, 6 mg/kg propofol (IV injection, Propofol; Fresenius Kabi, Tokyo, Japan) and isoflurane (Isoflur; DS Pharma Animal Health, Osaka, Japan) were administered, respectively. The anesthetized animals were held in the right-side recumbent position and a thoracotomy was performed at the left fourth intercostal region. A pacemaker lead electrode terminal, dipole electrode lead (TY216-033; UNIQUE Medical Co., Ltd., Tokyo, Japan), was fixed on the left atrium by suturing with 5–0 polypropylene sutures (Proline; Johnson Endo Johnson Co., Ltd., Tokyo, Japan). The intercostal region was closed in accordance with routine procedures, and the operation was completed after introducing the connecting terminal of the pacing lead with the main body of the pacemaker from the back region to outside the body. Animals were housed to enable them to rest for 10 days after the implantation surgery, considering the effect of the surgery. During this resting period, 0.2 mg/kg meloxicam (SC injection for 3 days, Metacam 0.5%; Nippon Boehringer Ingelheim Co., Ltd., Tokyo, Japan) and 5 mg/kg enrofloxacin (SC injection for 10 days, Baytril; Bayer Pharmaceutical, Osaka, Japan) were administered for analgesia and infection prevention, respectively. After the resting period, the pacing lead was connected to an external pacemaker and RAP (2 mV, 390 bpm) was started.

2.3. Blood samples

The NVAF animal model was subjected to general anesthesia and blood samples were collected. The animals were held in the right-side recumbent position and the skin was incised at the cervical region to expose the jugular vein. The jugular vein was held with a surgical support thread and slightly incised. A nutritional catheter (Atom nutritional catheter 5 Fr; Atom Medical International, Inc., Tokyo, Japan) was inserted into the jugular vein from the incised region. The catheter was led to the right atrium while confirming the tip under X-ray illumination, and an atrial blood sample was collected. A peripheral blood sample was collected from the lateral saphenous vein using a 20-G winged needle. Blood samples were collected before and 1, 2, and 3 weeks after the start of RAP. PT, APTT, and levels of Fibn, D-dimer, and antithrombin III (ATIII) were measured using the T-TAS and conventional blood coagulation tests.

2.4. T-TAS

Two different kinds of exclusive chip, the AR chip (for mixed white thrombus analysis) and the PL chip (for platelet thrombus analysis), were used in the T-TAS. The interior of the AR chip was coated with Type I-A collagen derived from pig tendon (Type I-A collagen) and thromboplastin derived from rabbit (rabbit tissue thromboplastin). By passing a blood specimen involving sodium citrate-treated whole blood to which calcium ions had been added into the AR chip at a flow rate of 10 μl/min and shear stress of 600 s^{-1}, thrombus formation was accelerated by the interaction between platelets and the blood coagulating system. A platelet-containing fibrin-rich thrombus was formed and the pressure inside the chip increased owing to the occlusion of the flow channel. The set-up for the PL chips (PL chip, chip for platelet thrombus analysis) differed as they were coated only with collagen. The procedure here involved flowing whole blood treated with hirudin (without citrate) into the PL chip at a flow rate of 18 μl/min and shear stress of 1500 s^{-1}. Platelets form blood clots that contain only platelets, which are produced by adhesion and cohesion through fibrinogen and VWF. These clots cause the pressure in the system to rise by closing the flow path. It is thus possible to evaluate the solidification state by continuously measuring the pressure rise in these chips [19–21]. Thus, flow pressure patterns reflect the thrombus formation process in the microchip. And also, the process of thrombus formation in the flow path of the AR and PL chip is continuously monitored using a video-microscope (10×) under the flow chamber (Fig. 1).

Thrombus formation-associated increases in the internal pressure of the AR and PL chips were measured after putting whole atrial and peripheral blood samples into the microflow channels of the chips. Clogging start time (T_{10}; the time required to reach internal pressure of +10 kPa from the baseline) and occlusion time (OT; the time required