Blood Coagulation Status during Cryofreezing Ablation and Effects of the Direct Anticoagulants Dabigatran and Edoxaban

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Summary

Cryoballoon ablation is an established catheter-based approach to treat atrial fibrillation (AF). However, thromboembolic events cannot be avoided during cryoaablation. There is little data regarding the blood coagulation status during freezing.

The thrombin antithrombin complex (TAT) and prothrombin fragment 1+2 (F 1+2) of patient blood were measured during cryoballoon application when the cryoballoon temperature reached the nadir in 63 AF patients. TAT was also measured from porcine blood during cryoballoon freezing in 5 pigs.

The TAT and F 1+2 increased from 6.60 ± 5.65 to 9.16 ± 7.28 ng/mL (P = 0.004) and from 279.6 ± 146.4 to 323.6 ± 169.1 pmol/L (P = 0.003) between the control and during freezing, respectively. The TAT increased from 0.46 to 0.87 ng/mL during freezing compared to that of pre-freezing (P < 0.05), and it returned to 0.39 ng/mL in 30 minutes after an intravenous edoxaban administration (N.S.).

Dabigatran failed to exert sufficient anticoagulant effects during cryofreezing. In contrast, intravenous edoxaban seemed to provoke anticoagulation effects under extreme low temperature circumstances.

Key words: Catheter ablation, Cryoballoon

Among the various techniques for catheter ablation of atrial fibrillation (AF), cryoenergy is an established therapeutic option to achieve pulmonary vein isolation due to its evidenced efficacy and safety profile. However, cerebral thromboembolic events could be provoked in 0.2-5.6% patients. Despite a novel method to lower the incidence of asymptomatic cerebral embolisms, its incidence could not be reduced well. The aim of this study was to identify other factors of embolic events associated with cryofreezing.

Methods

Patient population: A total of 63 patients who underwent AF ablation using a second-generation cryoballoon (CB) were included. Exclusion criteria were the presence of an intracavitary thrombus, uncontrolled heart failure, moderate or severe valvular disease, a left atrial diameter of > 55 mm, and contraindications to general anesthesia. All patients signed informed consent forms for the ablation procedure. No patient had taken any anti-platelet agents during this study. The study was approved by the Institutional Ethics Committee on Human and Experimental Research at our institution.

Preprocedural management: Before the procedure, all patients underwent 2-dimensional transthoracic echocardiography to assess the left ventricular function and to rule out any structural and/or valve disease. Cardiac computed tomography and transesophageal echocardiography were performed the day before the ablation to analyze the left atrium and pulmonary vein anatomy and to rule out intracardiac thrombus formation. For patients taking direct oral anticoagulants, our practice for giving anticoagulation was as follows: (1) all patients had taken direct oral anticoagulants (DOACs) for more than 4 weeks before the CB ablation (CBA). Ordinary DOACs were taken as usual on the first day after hospitalization. On the second day after hospitalization following the CBA performed on the same day, the DOACs (edoxaban, rivaroxaban, apixaban) except for dabigatran were discontinued in the morning and given in the evening after confirmation of hemostasis of the puncture sites, depending on the start time of the ablation procedure and their consciousness level. An unfractionated heparin was routinely administered during the CBA to maintain the activated clotting time (ACT) level > 300 seconds. From the morning of the third day, the same

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samples sheath) positioned in the right atrium, where the cryoballoon was located closest to the tip of the FlexCath.

DOACs as prescribed before the CBA were resumed. In cases in which DOACs were prescribed twice a day, such as dabigatran and apixaban, the anticoagulants in the evening of the first day were switched to dabigatran. On the second (CBA day) and third day, dabigatran was administered. From the fourth day, the same DOACs as prescribed before the CBA were resumed as reported previously. The dose of dabigatran on the day of the CBA was 150 mg twice daily, however, it was adjusted at the physician’s discretion considering the age, value of the creatinine clearance, and risk of bleeding. The doses of DOACs before and after the CBA were also decided according to the age, body weight, and creatinine clearance level by the attending physicians. All patients were scheduled to be discharged from the hospital on the fourth day.

Clinical cryoballoon ablation procedure and blood sampling: All ablation procedures were performed under general anesthesia. The CB was positioned in the right atrium, and freezing was started prior to introducing the CB catheter into the left atrium for the AF ablation. A FlexCath was advanced as close as possible to the CB, and a blood sample was collected and added to sodium citrate through the FlexCath when the CB temperature reached the nadir (Figure 1). The temperature of the blood obtained in this way was considered to be lowest in the heart chambers during freezing. Citrated blood was drawn and spun for 15 minutes at 2000 g at room temperature, and then the supernatant was spun for 5 minutes at 11,000 g at room temperature and stored at −70°C. Coagulation assays were then performed to measure the thrombin antithrombin complex (TAT) and prothrombin fragment 1+2 (F 1+2) levels. Measurement of the TAT was undertaken using the TAT test (Sysmex, Kobe, Japan), and the F 1+2 measurement was performed with commercial kits (Dade Behring Marburg GmbH, Marburg, Germany).

For the index ablation procedure, single transseptal punctures were performed after intravenous heparin of 5000 IU was administered; for the 12-Fr inner-diameter deflectable sheath and CB catheter. In addition, heparinized saline was continuously infused to maintain an activated clotting time of > 300 seconds through the Flex Cath (Medtronic, Minneapolis, MN, USA) during catheter manipulation in the left atrium (LA) in order to prevent any thrombus formation in the space between the FlexCath and CB shaft.

Experimental setting: Five swine weighing 32.6 ± 5.5 kg (range 24.6-39.4 kg) were anesthetized, and maintained with isoflurane 2.5% to 3.0% per the routine protocol. Use of all animals was approved by the IVTEC Institution Animal Care (Kobe, Hyogo Prefecture). A right femoral vein was punctured and cannulated to allow the CB introduction. The FlexCath was advanced as close as possible to the CB, and the blood sample was collected and added to sodium citrate through the FlexCath when the CB temperature reached at nadir. The blood sample was immediately separated and frozen for the measurement. Coagulation assays were then performed to measure only the TAT. The TAT was the only parameter that could be measured from the porcine blood. Edoxaban was administered intravenously in a bolus fashion (40 mg) followed by a continuous infusion (1 mg/kg/hour) during the plateau phase of the cryoballoon temperature. Coagulation assays were also performed to measure the TAT in the same fashion as prior to the edoxaban administration.

Statistical analysis: Data are presented as the mean ± standard deviation. Differences between groups were examined for the statistical significance using the Mann-Whitney U test. A P-value of less than 0.05 denoted the presence of a statistical difference.

Results

Study population: Of the total 63 patients enrolled with paroxysmal AF and referred for AF ablation, 48 (76%) were male, and the mean age was 63 ± 13 years. No patients presented with structural heart disease. The CHADS 2 score was calculated for each patient and was 0 in 41%, 1 in 49%, and 2 in 10%. A large 28-mm second generation CB was used in all cases.

Coagulation assays:

Clinical investigation: At baseline, the average coagulation parameters of the TAT and F 1+2 in all study patients were 6.60 ± 5.65 ng/mL and 279.6 ± 146.4 pmol/L, respectively, and they were significantly higher than the reference range due to the uninterrupted dabigatran administration. However, these two parameters significantly further became elevated to 9.16 ± 7.28 ng/mL (P = 0.004) and 323.6 ± 169.1 pmol/L (P = 0.003) during freezing, respectively (Figure 2). No injurious adverse effects were not provoked by this procedure.

Experimental investigation: At baseline, the average coagulation parameter of the TAT in all animals was 0.46 ± 0.021 ng/mL, and this increased during freezing to 0.87 ± 0.015 ng/mL (P < 0.05), and it returned to 0.39 ± 0.012 ng/mL by 30 minutes after the intravenous edoxaban as compared to the pre-freezing value (P = 0.27) (Figure 3).
Main findings of the present study: (1) The TAT and F1+2 significantly increased under an extremely low temperature and anticoagulation with dabigatran in humans. (2) Edoxaban was experimentally able to exert significant anticoagulation effects under the same extremely low temperature conditions.

Cryoballoon ablation has been demonstrated to be effective and safe for pulmonary vein isolation in patients with AF. However, strokes occurred in 5 out of 228 (2.2%) patients, whose CHADS2 score was less than 1. We previously reported that asymptomatic strokes were provoked by cryoballoon applications in approximately 20% of patients even under DOAC treatment. Symptomatic cerebral embolisms occurred during the cryoballoon ablation of AF in 0-0.5% of the patients. Procedure related asymptomatic strokes were detected in 4-27% of patients after the cryoballoon application for AF. The procedure in order to prevent procedure-related embolic
events during cryoballoon ablation has been assessed, however, such a procedure was able to reduce the incidence from 23.3 to 4.7%.

(3) remaining air attached to the thromboembolisms provoked by the activation of the coagulation cascade, and (3) remaining air attached to the instruments due to insufficient removal of air bubbles.

Among these factors, the activation of the coagulation cascade provoked by extremely low temperatures should be taken into account when we consider the precautions in terms of avoiding thrombotic events. Prophylactic procedures to avoid embolic events have been proposed during AF ablation. Uninterrupted warfarin, uninterrupted DOAC, and aggressive anticoagulation with a minimum ACT of 350 seconds have been recommended. Su, et al. recommended that a target ACT between 350 and 400 seconds is recommended during cryoballoon applications. This value is relatively higher than that for RF applications in terms of preventing thromboembolisms. Considering the enhanced coagulation status provoked by extremely low blood temperatures, the maintenance of a longer ACT time than 400 seconds could be recommended.

The reference interval for the TAT in dogs was found to be < 0.25 ng/mL, and there seems to be a large difference regarding the reference range of the TAT between humans and animals. An elevated TAT is recognized as a coagulation marker to detect a prothrombotic state. However, the specificity for thrombosis is better for F 1+2 than TAT, suggesting that F 1+2 may be the most useful marker for the earlier phase of thrombosis. In addition, F 1+2 directly reflects thrombin generation, and may be a very specific test for the coagulation activation. Using F 1+2, it is possible to measure hypercoagulable and hypo-coagulable states, and to detect the activation process in patients taking DOACs, which cannot be found by routine tests such as the INR and aPTT. In the present human study, both the F 1+2 and TAT levels significantly increased during cryofreezing, and strongly suggested a significantly higher coagulation status necessitating a more intense anticoagulation during cryofreezing. In the present animal study, only the TAT could be measured. It also demonstrated a significantly greater coagulable status during cryofreezing, and it could be converted to the pre-freezing level by intravenous edoxaban. Edoxaban could be expected to exert sufficient anticoagulation effects even under extremely low blood temperature conditions.

**Study limitations:** This study has several limitations. First, it was a single-center study. Second, the overall study population size was limited. Third, the study was a single arm, and there was no comparison to a control. Fourth, no follow-up of the study patients was undertaken, which precluded the long-term clinical implications. Fifth, the concentration of the TAT obtained from porcine hearts was very low, and such a level might not be applicable to clinical conditions. Sixth, to which extent the elevation of the TAT level is acceptable in terms of avoiding thromboembolic events during cryoablation requires further investigation. Finally, additional studies will clarify whether DOACs other than dabigatran will be able to provide sufficient anticoagulation effects under extremely low blood temperature conditions.

**Conclusion**

Dabigatran failed to exert sufficient anticoagulant effects during cryoballoon ablation. In contrast, intravenous edoxaban seemed to provoke anticoagulation effects under extremely low temperature conditions.

**Disclosure**

**Conflicts of interest:** None.

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