Prothrombin time tests for the monitoring of direct oral anticoagulants and their evaluation as indicators of the reversal effect

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Running Head: prothrombin time tests for monitoring DOACs

Abstract

Introduction: The prompt assessment and the reversal of direct oral anticoagulants (DOACs) are urgent matters in the emergency care setting. Thus, we planned to elucidate the adequate prothrombin time (PT) test for the evaluation of the anticoagulant effects of various DOACs.

Methods: The anticoagulant effects of rivaroxaban, apixaban and edoxaban were measured with three PT tests (Triniclot® PT Excel S, Neoplastin® R and Thromborel® S). Human plasma was spiked with each DOAC at a range of 0 to 1000 ng/mL, and the PT was measured using each PT test. In another series, the reversal effect of either 4-factor prothrombin complex concentrate (PCC) or activated prothrombin complex concentrate (aPCC) was evaluated with each PT test.

Results: All PT reagents correlated with the concentrations of each DOAC, however, the reactivity was considerably different between the DOACs and the PT tests. A prolonged PT with DOACs was reversed both by PCC and aPCC in a dose-dependent manner; however, Triniclot® PT Excel S showed re-prolongation of the PT with higher dose of PCC.

Conclusions: The proper choice of PT test is necessary for the assessments of the anticoagulant activity of DOACs. It is also important to understand the different characteristics of each PT test for the assessment of the reversal effects of PCC.

Keywords

prothrombin time; anti-factor Xa activity; rivaroxaban; apixaban; edoxaban

Introduction

Life-threatening bleeding such as massive and intracranial hemorrhage (ICH) during anticoagulant therapy with direct oral anticoagulants (DOACs) is a present danger. [1,2] It would be undoubtedly favorable if real-time monitoring for the anticoagulant activity of these agents were available.

The measurement of anti-factor Xa activity is a possible candidate, [3,4] however, this procedure is not consistently applicable in most of local laboratories. Instead, the prothrombin time (PT) test is more suitable if it is appropriately sensitive for practical use. The usefulness of PT tests for the monitoring of certain DOACs has been revealed in previous reports, [5,6] and thus, we planned the comprehensive comparison of various PT tests for all available DOACs in this study.

In the "Guidelines for the Management of Spontaneous Intracerebral Hemorrhage ~~(ICH)~~", [7] the American Heart Association Stroke Council recommended treatment with prothrombin complex concentrates (PCC) or activated PCC (aPCC) for the patients with ICH under the treatment of DOACs. However, there have been no randomized trials of reversing agents for DOACs among patients with ICH or other major bleeding complications. Since the reactivity of the PT test is reported to be different depending on the DOACs, we need to examine which PT test for each DOAC is most suitable as far as the reversal effect is concerned. We further tried to elucidate the characteristic difference of

various PT tests and their potentials as monitoring tests for the reversal effects of PCC and aPCC.

Materials and Methods

Spike test

Pooled citrated normal human plasma was spiked with either rivaroxaban, apixaban or edoxaban at increasing concentrations to assess the impact in the different kinds of PT tests. Rivaroxaban and Edoxaban were kindly donated by Bayer Healthcare (Wuppertal, Germany) and Daiichi Sankyo (Tokyo, Japan) respectively. Apixaban was purchased from Selleckchem (Houston, USA). Working solutions of 100, 300, 500,700 and 1000 ng/ml of each DOAC were obtained from the stock solution at a dose of 1.0 mg/DMSO 1.0 mL by mixing with pooled citrated normal human platelet-poor plasma.

PT test

The PT was measured with the following method: 50 μL of plasma samples were incubated at 37°C up to 60 s and mixed with 100 μL of calcium and thromboplastin. The different thromboplastin reagents used were Triniclot® PT Excel S (trade name in Japan: Shinplastin® Excel S, Kyowa Medex, Tokyo, Japan), Thromborel® S (Dade Behring, Newark, USA) and Neoplastin® R (Diagnostica Stago, Asnieres, France). Clotting time was measured on a COAGTRON® 350 (Kyowa Medex, Tokyo, Japan) for all PT tests.

Reversal effect of PCC

After each DOAC was incubated at final concentrations of 0, 100, 300, 500, 700 or 1000 ng/mL with the pooled plasma for 15 min, 4-factor PCC (PPSB-HT®, Nihon Pharmaceutical, Tokyo, Japan) was added at concentrations of 0, 0.63, 1.25, 2.5 or 5.0 U/mL, and these aliquots were incubated at 37°C. After 15 min of incubation, the PT was measured with three PT tests. For the comparison of the magnitude of reduction, reduction rate (RR) was calculated. RR = PT (PCC 0 – PCC 1.25) / PT (without DOACs and PCC)

Reversal effect of aPCC

Similar to PCC, each DOAC was incubated at final concentrations of 0, 100, 300, 500, 700 or 1000 ng/mL with the pooled plasma for 15 min, aPCC (FEIBA®, Baxter, Vienna, Austria) was added at concentrations of 0, 0.63, 1.25, 2.5 or 5.0 U/mL, and these aliquots were incubated at 37°C. After 15 min of incubation, the PT was measured with three PT tests. For the comparison of the magnitude of reduction, reduction rate (RR) was calculated. RR = PT (aPCC 0 – aPCC 1.25) / PT (without DOACs and aPCC)

Statistical analysis

Statistical analysis was performed using the STATVIEW® program (Abacus Concepts, Berkeley, CA, USA). The Pearson product-moment correlation coefficient was used to correlate the distribution of PT values between the PT reagents. Overall statistical significance among serial measurements was analyzed with a repeated measures ANOVA, followed by an individual comparison with the paired *t*-test as appropriate. $P < 0.05$ was considered statistically significant.

Results

Spike test

The spike tests were repeated three times and the same results were obtained. The summarized results of three independent experiments for each DOAC are depicted in Figure 1. A concentration-dependent prolongation of the PT was found in all DOACs at a concentration from 0 to 1000 ng/mL. The relationship between the PT and each DOAC concentration was almost linear for each PT test at the range of examined concentrations, and thus, slopes (slope of the linear regression line) and R^2 (coefficient of determination) were compared between the reagents. The slopes of correlations between concentrations of rivaroxaban and the PT were greatest in Neoplastin® and Triniclot® followed by Thromborel® ($p < 0.01$). The order was Triniclot® followed by Neoplastin® and Thromborel® in apixaban, and Triniclot® followed by Neoplastin® and then by Thromborel® in edoxaban (Table 1).

Reversal effect by PCC

The reversal effect of PCC was examined three times for each DOAC and each PT test, and the representative results are shown in Figure 2−4. A significant prolongation of the PT was observed in the samples pre-incubated with each DOAC from 0 to 1000 ng/mL without PCC. When PCC 1.25 U/mL was added to the peak therapeutic concentration of rivaroxaban (100 ng/mL)-spiked samples,[8] the PT decreased most prominently with

Neoplastin® and was reduced from 19.7 s to 13.6 s (RR, 0.45) (Table 1). In the case of Triniclot®, the PT decreased at PCC concentrations between 0.63 and 1.25 U/mL, (RR, 0.24) then the PT started to prolong until PCC dose of 5.0 U/mL. The PT at 5.0 U/mL of PCC was 26.4 s and longer than the original PT (20.4 s [PCC 0 U/mL]). Thromborel® and Neoplastin® did not show such re-prolongation and the PT reached a plateau when the PCC dose was 2.5 U/mL. In case of the therapeutic concentration of apixaban (approximately 100 ng/mL), [9] the PT measured with Triniclot® decreased from 17.6 s (PCC 0 U/mL) to 13.6 s (1.25 U/mL) and the RR was 0.28. Similarly, 1.25 U/mL of PCC reduced the PT by 4.7 s (RR, 0.35) and 3.1 s (RR, 0.25) in Neoplastin® and Thromborel®, respectively. In case of the therapeutic concentration of edoxaban (approximately 300 ng/mL), [10] the PT reduced by 6.0 s (RR, 0.48), 8.2 s (RR, 0.61) and 4.2 s (RR, 0.35) in Triniclot®, Neoplastin® and Thromborel®, respectively.

Reversal effect by aPCC

The examination for the reversal effect of aPCC was also repeated three times for each NOAC and each PT test, and the representative results are shown in Figure 5−7. In a similar manner to PCC, the PT prolonged most with 100 ng/mL of rivaroxaban in Neoplastin® and Triniclot®, and 1.25 U/mL of aPCC reduced the PT measured by Neoplastin® from 19.7 s to 10.8 s with a RR of 0.66. The RR was 0.44 in Triniclot® and 0.32 in Thromborel®. Regarding the re-prolongation of the PT recognized in PCC, such a phenomenon was not seen in any of the PT tests. The PT reached a plateau at aPCC dose of 2.5 U/mL, and all

three PT tests demonstrated similar trends. The changes in the PT were smaller in apixaban in all PT tests and when the apixaban concentration was 100 ng/mL, the RR was 0.35 in Triniclot®, 0.43 in Neoplastin® and 0.31 in Thromborel®. In case of an edoxaban concentration of 100 ng/mL, 1.25 U/mL of aPCC reduced the PT by 0.60, 0.73, and 0.32 in Triniclot®, Thromborel® and Neoplastin®, respectively.

Discussion

The present study showed that there was a good linear correlation between spiked DOAC concentration and the PT value of the three PT reagents. This study also revealed that there were considerable differences among the PT reagents in the magnitude of responses to DOACs. The reactivity for rivaroxaban would be sufficient in Neoplastin® and Triniclot®, but not in Thromborel®. In case of edoxaban, the reactivity would be acceptable in Triniclot®, but would not be sufficient in Thromborel®. In the steady state of rivaroxaban therapy for the stroke prevention in non-valvular atrial fibrillation (20 mg once daily), the peak plasma concentration at 3 hour after intake of rivaroxaban was reported to be ranging from 159.6 to 359.8 ng/ml.[9] Though significant prolongation of the PT was recognized with this range, considerable variability among the PT reagents existed. Thus, we have to consider which PT tests are most suitable for the detection of activity. We examined three popular PT tests and Neoplastin® and Triniclot® would be clinically useful for the monitoring of rivaroxaban, and Triniclot® will be suitable for edoxaban. As for apixaban,

Dale et al. [6] reported that Triniclot® showed moderate reactivity (slope = 0.24, R^2 = 0.993) to apixaban. Our measurement also resulted in a similar result (slope = 0.37, R^2 = 0.999), and we speculated the Triniclot® might be useful to detect the apixaban dose. On the other hand, Douxfils et al. [11] reported that PT tests were not sensitive enough to evaluate the activity of apixaban. Kanemoto et al. [12] reported that PT values measured by Triniclot® after 5 mg intake were 15.4 ± 1.7 s at the peak. Since the reference value of Triniclot® is 12.7 s to 15.2 s and the mean peak PT is just above the reverence value, it might be difficult to detect the anticoagulant activity as a prolonged PT. This issue should further be examined in the clinical practice.

According to the sensitivity, the peak DOAC level after the standard dose (rivaroxaban, 20 mg once daily; apixaban, 5 mg twice daily; edoxaban, 60 mg once daily) intake can be measurable by adequate PT tests in cases of rivaroxaban and edoxaban. However, since the reported trough level of rivaroxaban was 22.3 ng/mL, [13] all PT tests may not be sensitive enough to detect this low range.

Though not all PT tests are sufficiently sensitive, we think that monitoring with the PT is still valuable. The incidences of serious bleeding are reportedly 2.12%/year when rivaroxaban was administered at 20 mg/day, [14] 1.27% when 5 mg of apixaban was administered twice daily [15] and 2.75%/year when edoxaban was administered at 60 mg/day [16], and it was also reported that proper dosing decreases the fatal events. [17] Since the activity of DOACs peaks at 1 to 3 h, [18] we recommend that the peak PT value should be

checked with a proper test for the effective and safe control. In addition, the measurement of the PT will be extremely useful in the emergency setting. Except for the developing of specific antidotes, [19-21] it has been widely accepted that PCC or aPCC are efficacious for the reversal of DOACs, [22-25] and administration of PCC 25-50 U/kg or aPCC 30-50 U/kg has been generally recommended if an overdose was suggested. [25] Thus, it will be favorable if the PT could be used to the necessity of the treatment and could reflect the reversal effects of prohemostatic agents. Several reports have reported the usefulness of PT tests for those purposes, [26] however, only a few studies have compared the performance of different PT tests and the results are inconsistent. [27,28]

PCC contains inactive factor II, IX, X and VIIa and aPCC contains activated factor VII and factors II, IX, X. [29] The American Heart Association guidelines recommended the use of PCC or aPCC as a potential reversal agent.[7] In the present study, the PT measured with Triniclot® decreased dose dependently from a PCC dose of 0.63 U/mL to 1.25 U/mL, and then the PT started to prolong when the PCC dose was over 2.5 U/mL. This phenomenon was estimated to reflect the effects of heparin added to PCC. PCC and aPCC were originally developed for the replacement of vitamin K-dependent clotting factors. Since the thrombotic adverse events have been reported as a major adverse event, [30] heparin was added to the second-generation PCCs. For example, PPSB-HT® contains 0.25 U of heparin sodium in 1 U of PCC. Then, does this heparin interfere with hemostasis? We think the phenomenon was observed only in an *ex vivo* study. The anticoagulant effect of heparin

may not be of concern in practical use because the recommended PCC dose is 50 U/kg maximal (approximately 1 U/mL).

Interestingly, the re-prolongation effect of PCC was observed only in the Triniclot®. This is because other PT tests contain protamine to reverse heparins. Thus, we think Triniclot® is more suitable for evaluating the net reversal effect of PCC.

In summary, a linear and dose dependent prolongation of PT was recognized in cases of rivaroxaban and edoxaban at the therapeutic range. Triniclot® will be the most appropriate PT test for the monitoring of DOACs. For the evaluation of the reversal effects of heparin-containing PCC, we think Triniclot® is more suitable than the others. After all, it is important to keep it in mind that there are considerable differences between the PT tests.

Finally, there are some limitations in this study. First, we examined the PT in relatively high-concentration DOACs to examine the reversal effects in overdose cases. However, the PT in lower concentrations of DOACs needs to be examined to evaluate the monitoring effect in daily practice. Second, we examined only a limited number of PT tests in the present study. Since considerable differences were revealed in this study, physicians need to recognize the reactivity and the characteristic of PT tests used in their own facilities. Finally, since each PT test showed linear correlation between the concentration and PT, it would be more favorable if the data could be comparable by simple equation. We also have to confirm that despite the linear correlations at all concentrations between drug level and PT, they still are not suitable for routinely monitoring of these drugs.

Conclusion

PT tests will not be suitable for the routine monitoring of DOACs, however, they could be useful in certain occasions such as overdose, complicating the critical bleeding and considering the treatment with PCC or aPCC. However, since significant variability exists, physicians should be aware of the sensitivity of their own assays to each DOACs. PT tests such as Triniclot® and Neoplastin® will be the useful assays to measure pharmacodynamic effects of rivaroxaban and edoxaban in the emergency care setting. The reversal effects of PCC and aPCC could also be evaluated by proper PT tests. It is therefore suggested that PCC and aPCC could better be used for emergency reversal of DOACs under monitoring by these PT tests.

Declaration of Conflicting Interests

Emmi M works for Kyowa Medex Co. Ltd. The other authors state that they have no conflict of interests.

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Legends

Figure 1: Impact of direct oral anticoagulants on the prothrombin time (PT).

Direct oral anticoagulants prolonged the PT concentration-dependently. The relationship was almost linear in all PT tests and the magnitude of responses to DOAC depended on the PT reagent. The response ranged from 0 to 1000 ng/ml.

◆: Triniclot® Excel S (blue), ▲: Neoplastin® R (green), ■: Thromborel® S (red)

Figure 2: Impact of prothrombin complex concentrate (PCC) on the prothrombin time (PT) under the use of rivaroxaban.

A concentration-dependent decrease of the PT was recognized after PCC administration. In case of Triniclot® Excel S, the PT decreased in a dose-dependent manner at a PCC dose between 0.63 and 1.25 U/mL. The PT started to prolong again when the PCC dose was over 2.5 U/mL or more. Such re-prolongation was not observed in Neoplastin® R and Thromborel® S, where the PT reached a plateau at the PCC concentration of 2.5 U/mL.

Figure 3: Impact of prothrombin complex concentrate (PCC) on the prothrombin time (PT) under the use of apixaban.

A concentration-dependent decrease of PT was recognized after PCC administration. In case of Triniclot® Excel S, the PT decreased in a dose-dependent manner at a PCC dose between 0.63 and 1.25 U/mL. The PT started to prolong again when PCC dose was over

2.5 U/mL or more. Such re-prolongation was not observed in Neoplastin® R and Thromborel® S, where the PT reached a plateau at the PCC concentration of 2.5 U/mL.

Figure 4: Impact of prothrombin complex concentrate (PCC) on the prothrombin time (PT) under the use of edoxaban.

A concentration-dependent decrease of the PT was recognized after PCC administration. In the case of Triniclot® Excel S, the PT decreased in a dose-dependent manner at a PCC dose between 0.63 and 1.25 U/mL. The PT started to prolong again when the PCC dose was over 2.5 U/mL or more. Such re-prolongation was not observed in Neoplastin® R and Thromborel® S, where the PT reached a plateau at the PCC concentration of 2.5 U/mL.

Figure 5: Impact of activated prothrombin complex concentrates (aPCC) on the prothrombin time (PT) under the use of rivaroxaban.

A concentration-dependent decrease of PT was recognized after aPCC administration. When 0.63, 1.25 or 2.5 U/mL of aPCC was added to the plasma samples that had been spiked with rivaroxaban, each PT test showed the decrease of the PT in a dose-dependent manner. However, a further 5.0 U/mL of aPCC did not add any further effect.

Figure 6: Impact of activated prothrombin complex concentrates (aPCC) on the prothrombin time (PT) under the use of apixaban.

A concentration-dependent decrease of PT was recognized after aPCC administration. When 0.63, 1.25 or 2.5 U/mL of aPCC was added to the plasma samples that had been spiked with rivaroxaban, each PT test showed the decrease of the PT in a dose-dependent manner. However, a further 5.0 U/mL of aPCC did not add any further effect.

Figure 7: Impact of activated prothrombin complex concentrates (aPCC) on the prothrombin time (PT) under the use of edoxaban.
A concentration-dependent decrease of the PT was recognized after aPCC administration. When 0.63, 1.25 or 2.5 U/mL of aPCC was added to the plasma samples that had been spiked with rivaroxaban, each PT test showed the decrease of PT in a dose-dependent manner. However, a further 5.0 U/mL of aPCC did not add any further effect.

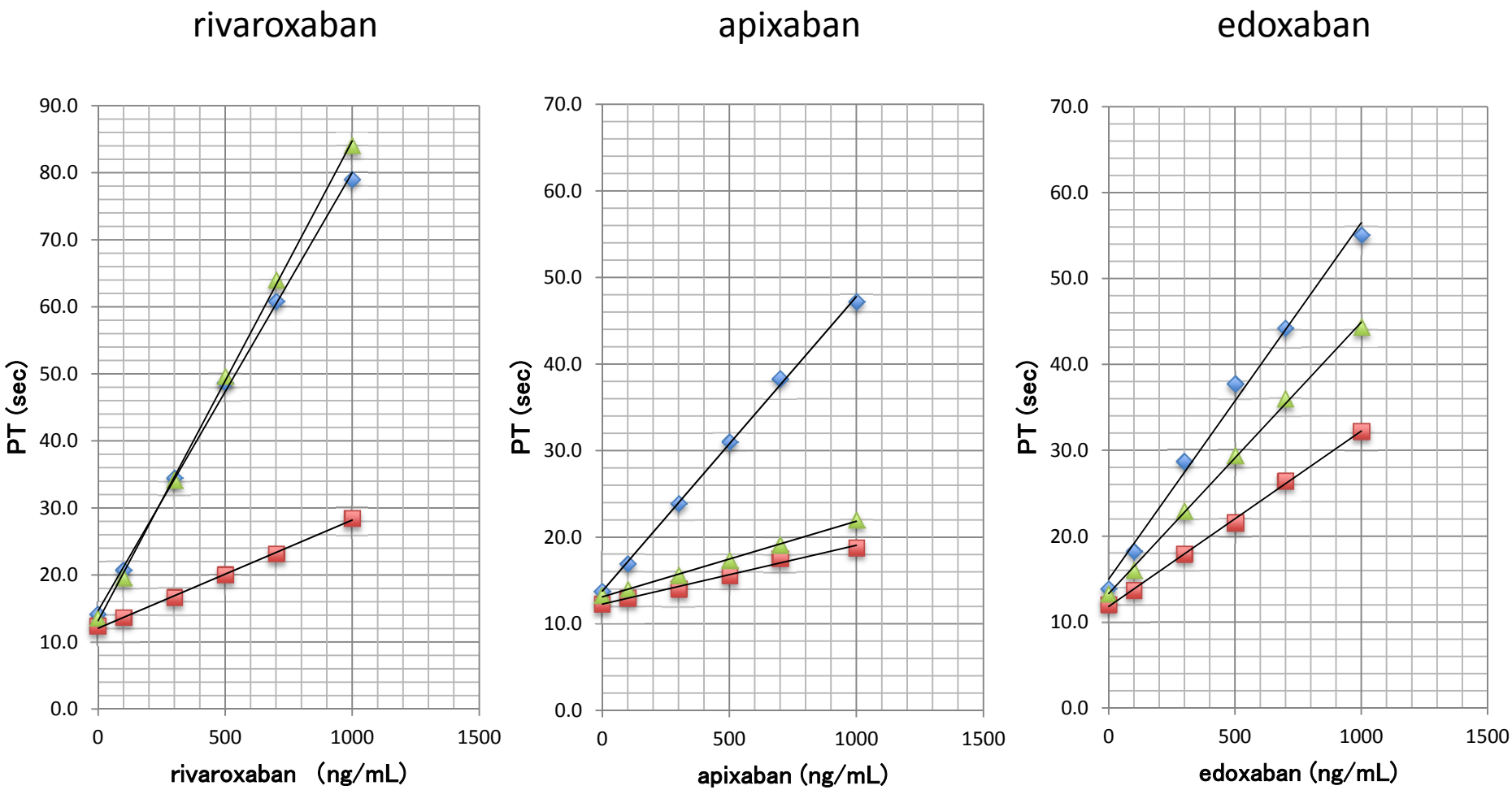
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Fig. 1

rivaroxaban

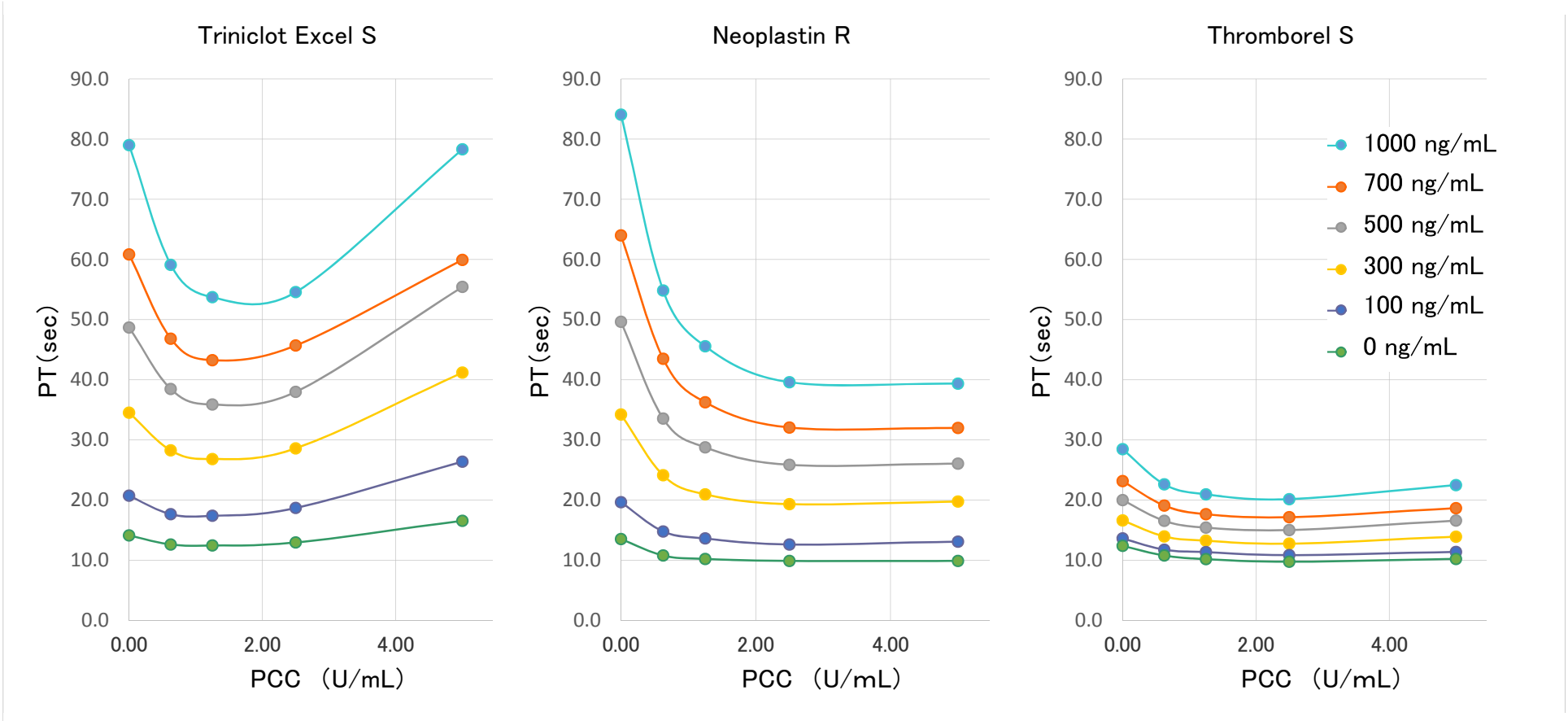

Fig. 2

apixaban

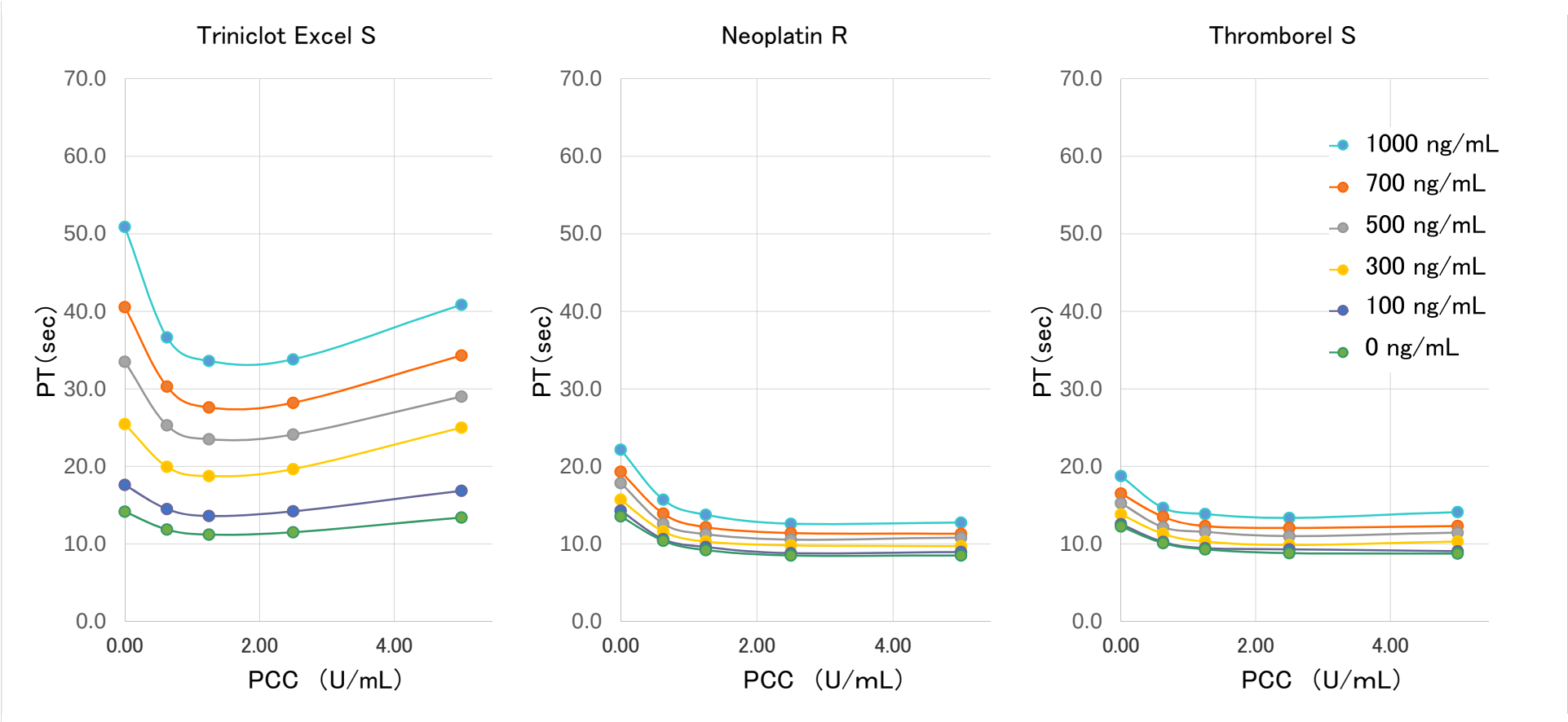

Fig. 3

edoxaban

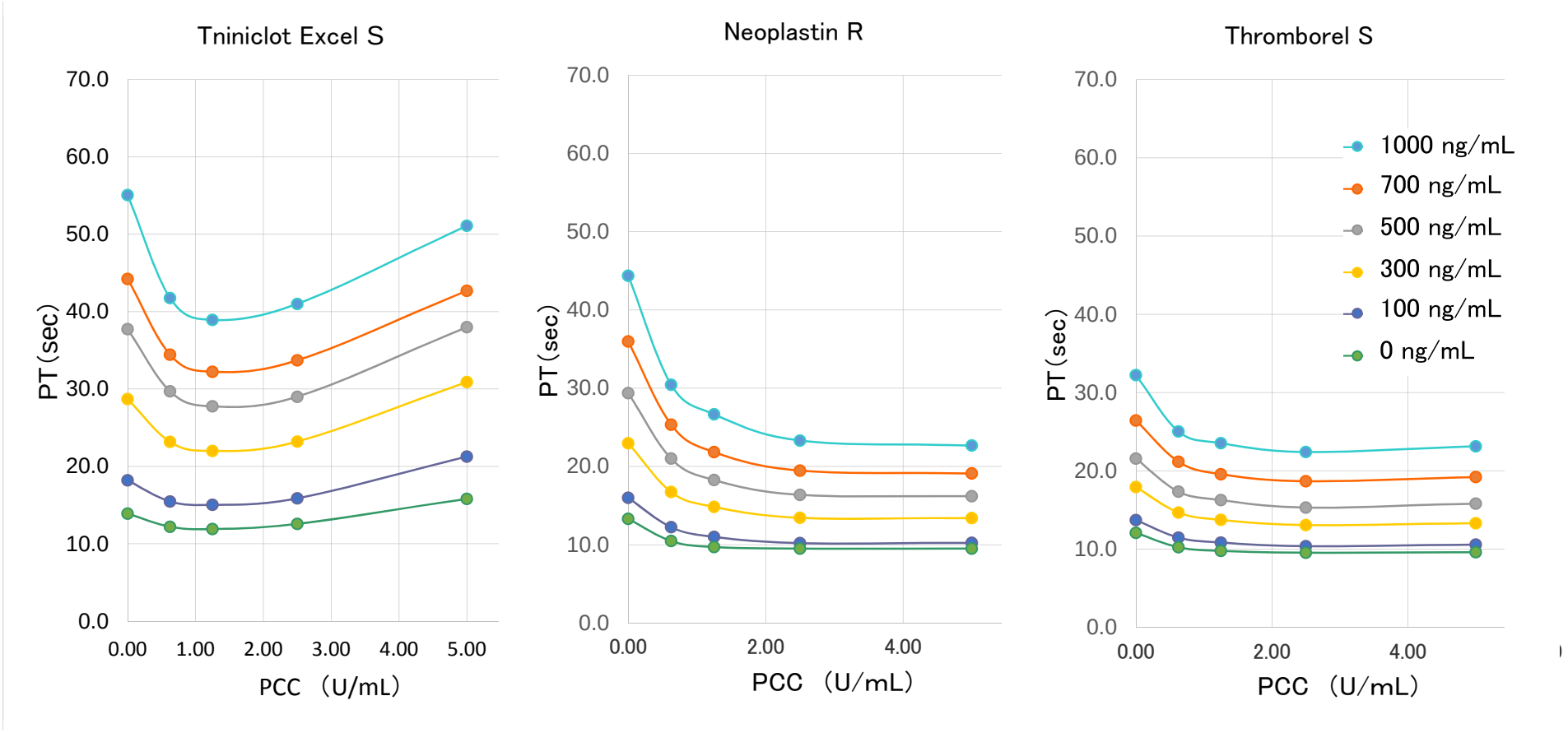

Fig. 4

rivaroxaban

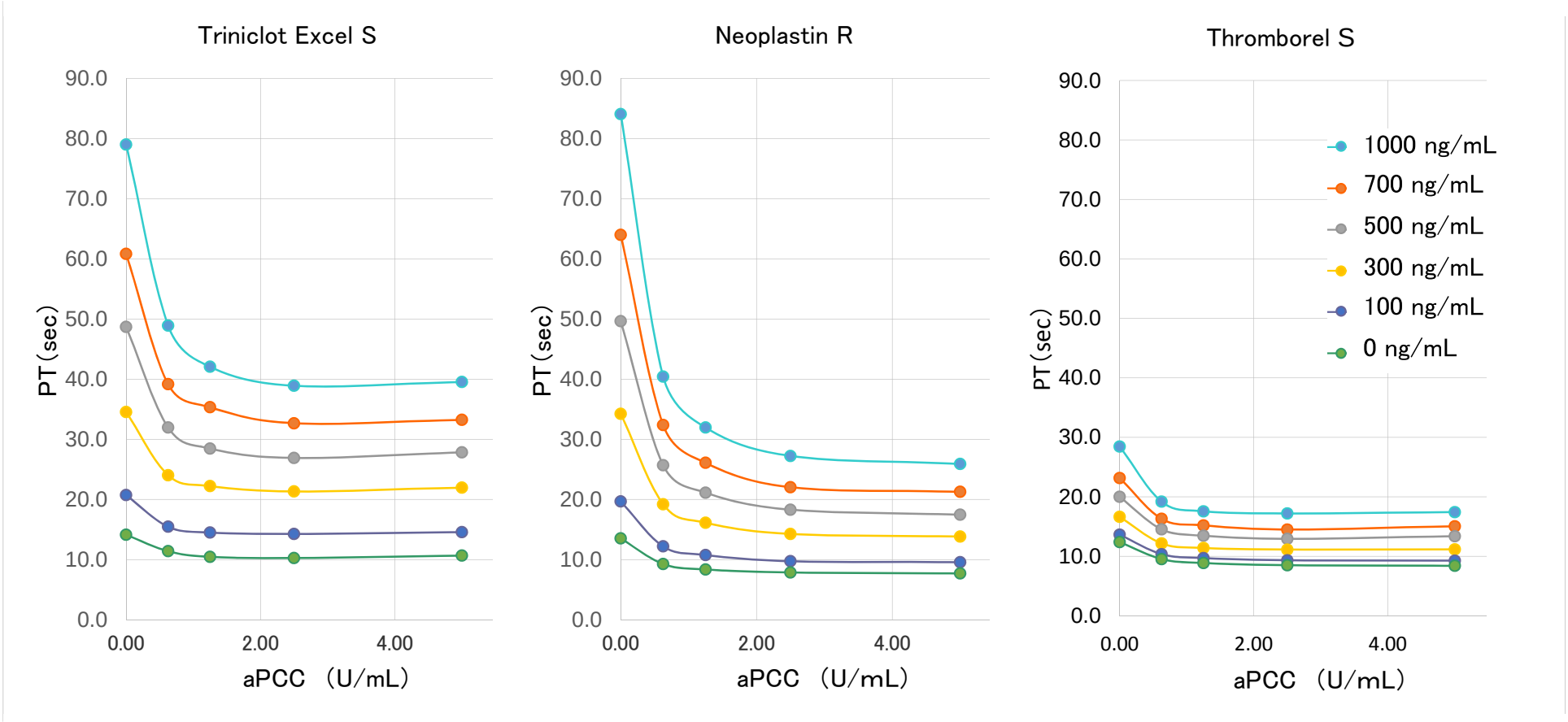

Fig. 5

apixaban

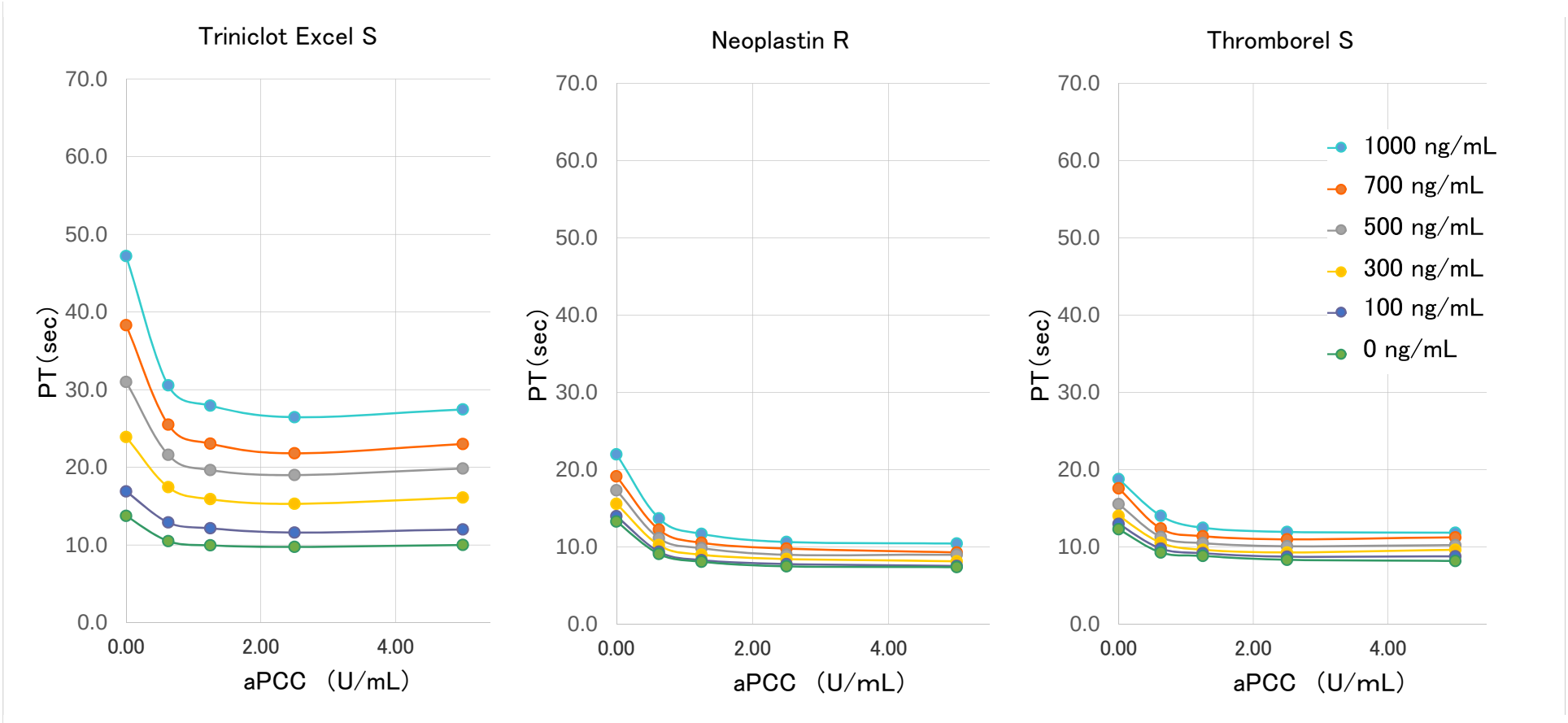

Fig. 6

edoxaban

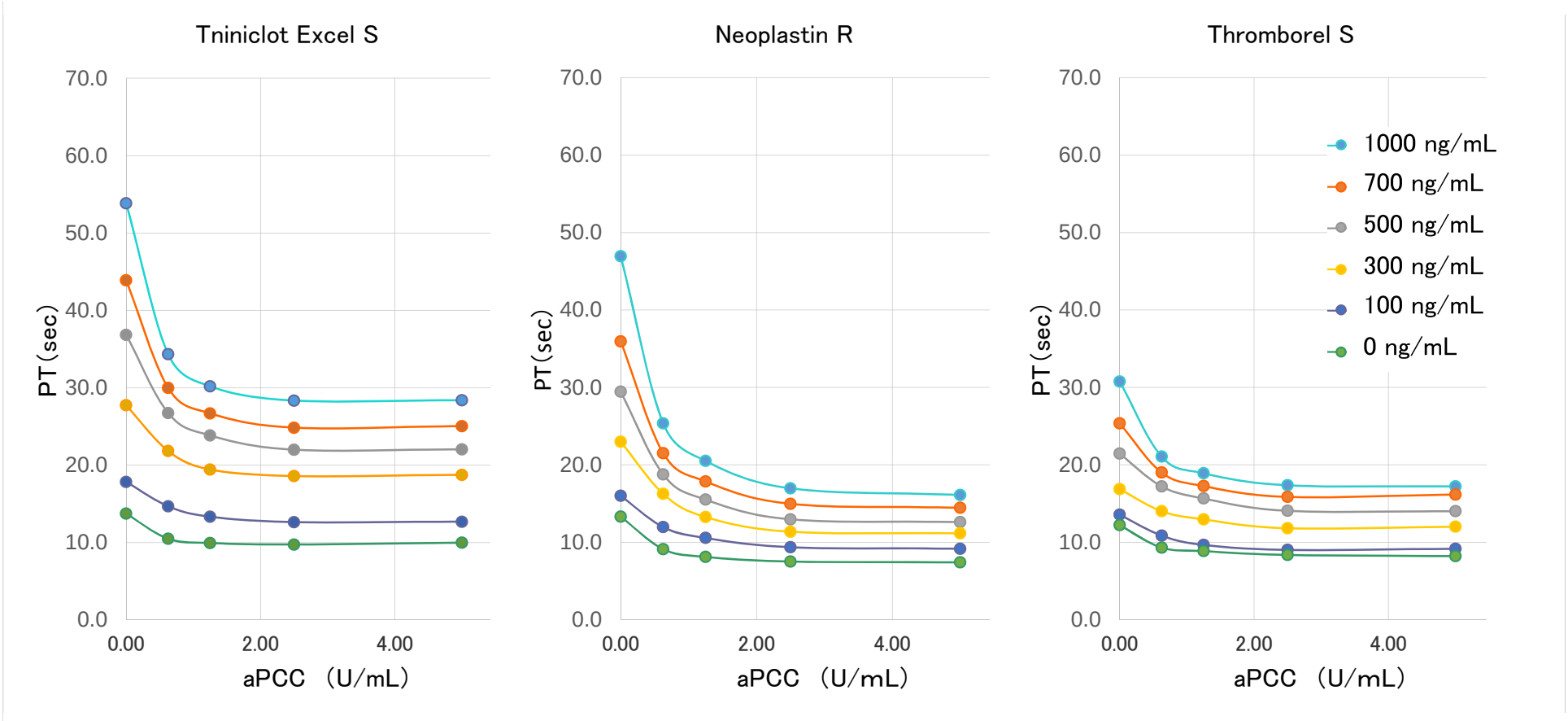

Fig. 7

Table 1 Prothrombin time reagents evaluated

Rivaroxaban (100 ng/mL[7])

PT reagent	Reference value (s)	Slope	R^2	Reduction rate (PCC)	Reduction rate (aPCC)
Triniclot PT Excel S	12.7 − 15.2	0.65	0.999	0.24	0.44
Neoplastin R	13.0 – 17.7	0.72	0.999	0.45	0.66
Thromborel S	9.8 – 12.1	0.16	0.998	0.18	0.32

Apixaban (100 ng/mL[29])

Triniclot PT Excel S	12.7 − 15.2	0.37	0.999	0.28	0.35
Neoplastin R	13.0 – 17.7	0.09	0.996	0.35	0.43
Thromborel S	9.8 – 12.1	0.07	0.995	0.25	0.31

Edoxaban (300 ng/mL[30])

Triniclot PT Excel S	12.7 − 15.2	0.41	0.991	0.48	0.60
Neoplastin R	13.0 – 17.7	0.32	0.999	0.61	0.73
Thromborel S	9.8 – 12.1	0.02	0.999	0.35	0.32

Slope ; slope of the linear regression line
R^2 ; coefficient of determination
Reduction rate (PCC) ; PT (PCC 0 – PCC 1.25) / PT (without DOACs and PCC)
Reduction rate (aPCC) ; PT (aPCC 0 – aPCC 1.25) / PT (without DOACs and aPCC)