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How the Direct Oral Anticoagulant Apixaban Affects Thrombin Generation Parameters

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ABSTRACT

Background and objectives: Apixaban is a direct oral anticoagulant (DOAC) targeting factor Xa and thus quenching thrombin generation and clot formation. However, little information is available on the influence that apixaban may have on the parameters of thrombin generation.
Methods: Aliquots of a pooled normal plasma have been added with increased concentrations of purified apixaban and were used to assess the degree of modification brought about by the drug on the basic tests of coagulation prothrombin and activated partial thromboplastin time (PT and APTT) and on thrombin generation parameters.
Results: The study shows that while apixaban has little effect on PT or APTT it does affect all the parameters of thrombin generation, including the lag-time (which is increased), the endogenous thrombin potential (ETP) and thrombin-peak (both decreased although to a different extent), and the velocity index (decreased). Interestingly, the above effects were more pronounced when the measurements were recorded in the presence of thrombomodulin, thus making the ratio (with/without thrombomodulin) to decrease consistently as a function of the apixaban concentrations.
Conclusions: These findings support the antithrombotic properties of apixaban and can help to understand the mechanism(s) of action of this drug. Thrombin generation could be used as a convenient laboratory tool to assess the anticoagulant activity of other drugs and to make between-DOAC comparison.

Introduction

Direct oral anticoagulants (DOAC) target specific coagulation factors (either thrombin or factor Xa) and because of this effect they have been reported to affect thrombin generation tests [1–5]. Although studies investigating the effect of apixaban on parameters of thrombin generation have been carried out [3] out scanty information is, however, available [6,7] for this direct activated factor X (FXa) inhibitor that has been licensed for the treatment and prophylaxis of venous thromboembolism [8] and for the prevention of stroke and systemic embolism in patients with atrial fibrillation [9]. We report here results on the most important parameters stemming from the thrombin generation curve carried out for a set of plasmas prepared by adding a pooled normal plasma (PNP) with increasing amounts of purified drug. The results may help to assess the effect of the drug on thrombin generation in comparison to such basic coagulation tests as the prothrombin and activated partial thromboplastin times (PT and APTT) which are poorly responsive to the effect of apixaban (10-12).

Material and Methods

Test Plasmas

Aliquots of blood were drawn from 20 healthy individuals and mixed with 1/10 trisodium citrate 109 mM in vacuum plastic tubes (Vacutainer, Becton Dickinson, Plymouth, UK). Specimens were centrifuged for 20 minutes at 2,880 g (controlled room temperature). Equal portions of plasma from each subject were mixed within one hour in a plastic container to prepare the pooled normal plasma (PNP) that was quickly frozen in liquid nitrogen and stored at -70 °C. On the day of preparation of the test plasmas, the PNP was thawed by immersion in a water bath at 37 °C and after repeated inversions was divided in eight aliquots labeled as 0, 10, 20, 50, 100, 200, 500 and 1,000 ng/mL. Purified (lyophilized) apixaban, obtained from Pfizer (New York, NY), was reconstituted in dimethyl sulfoxide at the final concentration of 1 mg/mL and the stock solution was diluted in PBS to obtain concentrations

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correspondent to 0, 1, 2, 5, 10, 20, 50 and 100 μg/mL (intermediate concentrations). Finally, test plasmas have been prepared by diluting 1:100 each of the intermediate concentrations with the PNP to get final apixaban concentrations correspondent to 0, 10, 20, 50, 100, 200, 500 and 1,000 ng/mL. Each of the eight test plasmas has been aliquoted in plastic tubes (1 mL each), snap frozen in liquid nitrogen and stored at - 70 °C. The process from thawing the PNP to freezing the aliquots of the test plasmas lasted no more than forty minutes. Testing for thrombin generation was carried out within two months from preparation. Plasmas were tested for each parameter in quadruplicate on four independent working sessions and the mean values used for statistical evaluation. A single lot of reagent for each method was used throughout the study.

Methods

Basic Tests

PT and APTT were measured by means of Recombiplastin 2G and Thrombosil APTT, respectively (Instrumentation Laboratory, Orangeburg, NY) on the automated coagulation analyzer ACL Top (Instrumentation Laboratory, Bedford, MA). Results were expressed as clotting time (patient-to-normal) ratio taking as normal the value obtained for the laboratory PNP.

Thrombin Generation Test

This was assessed according to Hemker et al. [13] as described with an home-made method [14]. Testing was based on the activation of coagulation after addition to plasma of such triggers as human recombinant tissue factor (1 pM) (Recombiplastin, Instrumentation Laboratory) and synthetic phospholipids (1.0 μM) (Avanti Polar, Alabaster, Alabama). Testing was repeated in another plasma aliquot after addition of rabbit thrombomodulin (Haematologic Technologies, Inc, VT) (4nM). Registration of thrombin generation was obtained with a fluorogenic substrate (Z-Gly-Gly-Arg-AMC HCl, Bachem, Bubendorf, Switzerland) (617 μM) by means of a fluorometer (Fluoroskan Ascent®, Thermo-Labsystem, Helsinki, Finland). Readings were recorded and calculated with a dedicated software (Thrombinoscope™, Thrombinoscope BV, Maastricht, The Netherlands), which displays thrombin generation curves and calculates the area under the curve, defined as endogenous thrombin potential (ETP) and expressed as nM thrombin times minutes (nM•min). Such other parameters stemming for the software as the time (minutes) of the lag-phase that follows the addition of the trigger and the initiation of thrombin generation; the thrombin peak (nM); the velocity index (VI) defined as VI = [Peak height/(time to peak – lag time)] were also recorded and analyzed. Results were also expressed as ETP-ratio or peak-ratio (i.e., the ratio of ETP or peak measured in the presence of thrombomodulin to the ETP or peak measured in its absence). This ratio represents the resistance to the anticoagulant action of thrombomodulin and can be taken as an index of procoagulant imbalance (the higher the ratio, the greater the procoagulant imbalance). To minimize analytical variability the entire set of test plasmas were analyzed within the same working session.

Statistical Analysis

Results for each parameter of thrombin generation and each test plasma were calculated and expressed as absolute values or as the ratio dividing their value by that obtained for the test plasma at 0 ng/mL apixaban. These ratios were then plotted against the respective apixaban concentrations. Finally, the ratios obtained dividing the value obtained at 200 ng/mL by the value obtained at 0 ng/mL apixaban concentrations were chosen to assess the degree of effect brought about by apixaban on each thrombin generation parameter. Ratios higher or lower than the unity indicate positive or negative effect, respectively.

Results

PT and APTT

PT- and APTT-ratios (patient-to-normal) were barely affected by apixaban; the PT-ratio being higher than the upper limit of the local reference range only when apixaban concentrations were greater than 200 ng/mL (Fig. 1). The APTT-ratio was still within the normal reference range when the concentration of apixaban was 400 ng/mL (Fig. 1).

Thrombogram

The area under the thrombin generation curve for plasmas added with increasing concentrations of apixaban are in Fig. 2. There was a concentration dependent decrease of the thrombogram both in the absence or presence of thrombomodulin.

Lag-Time

The absolute values of the lag-time are in Table 1. They increased with the apixaban concentration; the effect was more pronounced when the test was run in the presence than in the absence of thrombomodulin (Fig. 3A). The lag-time observed at 200 ng/mL increased to 2.53-times or 3.65-times compared to the value observed at 0 ng/mL when the test was run in the absence or in the presence of thrombomodulin, respectively (Table 2).

Endogenous Thrombin Potential (ETP)

The absolute values of ETP are in Table 1. In general, they decreased with the increasing apixaban concentrations. The effect was more pronounced when the test was run in the presence than in the absence of thrombomodulin (Fig. 3B). Moreover ETP in the presence of thrombomodulin decreased sharply up to 200 ng/mL apixaban and slowly afterwards (Fig. 3B). The ETP observed at 200 ng/mL decreased

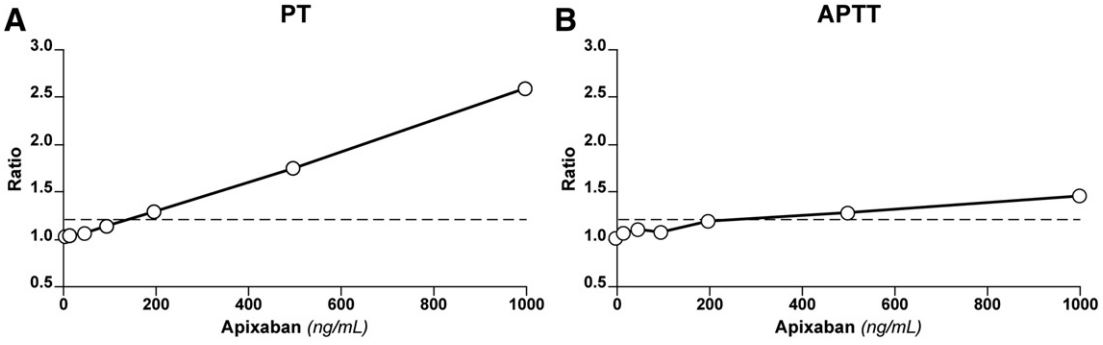

Fig. 1. Effect of increasing concentrations of apixaban on prothrombin and activated partial thromboplastin times (PT and APTT). Results are reported as the ratio obtained dividing the value corresponding to each apixaban concentration by the value obtained at 0 ng/mL. Horizontal broken lines represent the upper limit of the reference range.