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Full Length Article

Edoxaban, a direct factor Xa inhibitor, suppresses tissue-factor induced human platelet aggregation and clot-bound factor Xa in vitro: Comparison with an antithrombin-dependent factor Xa inhibitor, fondaparinux☆

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ABSTRACT

Introduction: Tissue factor-induced platelet aggregation and factor Xa (FXa) activity bound to clot contribute to the formation and growth of thrombus. The effects of edoxaban, a direct FXa inhibitor, on these responses were determined and compared with that of fondaparinux, an antithrombin-dependent (indirect) FXa inhibitor.
Material and methods: Human platelet aggregation was induced by human tissue factor (Dade Innovin or RecombiPlasTin) in platelet-rich plasma spiked with edoxaban or fondaparinux. Clot formed from human whole blood was incubated with 0.9 μM prothrombin in the absence or presence of FXa inhibitors. As the index of FXa activity, the amount of prothrombin fragment F1 + 2 was measured with an ELISA. Free FXa activity was measured using human FXa and its chromogenic substrate S-2222.
Results: Edoxaban inhibited tissue factor-induced platelet aggregation in a concentration-dependent manner with the IC_{50} values of 150 and 110 nM for Dade Innovin and RecombiPlasTin-induced platelet aggregation, respectively. At 1 μM, edoxaban completely inhibited the aggregation. Fondaparinux inhibited RecombiPlasTin-induced aggregation with the IC_{50} of 9.3 μM, but did not show complete inhibition up to 30 μM and had no effect on Dade Innovin-induced aggregation. Edoxaban inhibited both free and clot-bound FXa with the IC_{50} of 2.3 and 8.2 nM, respectively. Fondaparinux inhibited free FXa (IC_{50} 5.4 nM), but 40-times higher concentration were required to inhibit clot-bound FXa (IC_{50} 217 nM).
Conclusions: Edoxaban, a direct FXa inhibitor, was a more potent inhibitor of tissue factor-induced platelet aggregation and clot-bound FXa than fondaparinux, an indirect FXa inhibitor.

1. Introduction

In the process of thrombus formation, two main pathways, blood coagulation and platelet aggregation, play pivotal roles [1,2]. Therefore anticoagulants and antiplatelet agents are used to block these pathways for the prevention and treatment of thrombotic diseases. The blood coagulation and platelet aggregation do not act independently and there are cross talks between the two pathways. For example, thrombin, which is a final product of the coagulation pathway and forms fibrin, is a physiological platelet agonist and induces platelet aggregation via its platelet receptors, PAR-1 and PAR-4 [3,4]. Both thrombin-induced fibrin formation and thrombin-induced platelet aggregation contribute to the thrombus formation.

During the activation of the coagulation cascade, factor Xa (FXa) forms a complex with activated factor V and calcium ion on the surface of platelet membrane (so called prothrombinase complex) and converts prothrombin to thrombin [5,6]. Selective FXa inhibitors do not directly inhibit platelet aggregation mediated by thrombin [7,8], but by inhibiting FXa, FXa inhibitors are expected to suppress thrombin generation and has the potential to decrease indirectly thrombin-mediated platelet aggregation. Actually direct FXa inhibitors, apixaban and rivaroxaban, inhibit tissue factor-induced platelet aggregation in vitro [9,10]. In contrast, it is reported that FXa incorporated into the prothrombinase complex is highly protected from inhibition by fondaparinux, an antithrombin-dependent FXa inhibitor [11,12]. This raised a possibility that the inhibitory effect of fondaparinux on tissue factor-induced platelet aggregation would be limited.

FXa binds to clots during clot formation and contributes to the procoagulant activity of thrombi to grow thrombus [13–17]. When

Abbreviations: $AUC_{5\ min}$, area under the curve of 5 min-aggregation response; DMSO, dimethyl sulfoxide; F1 + 2, prothrombin fragment F1 + 2; FXa, factor Xa; IC_{50}, 50% inhibition concentration; PPP, platelet-poor plasma; PRP, platelet-rich plasma; SEM, standard error of mean; TBS, Tris buffered saline.

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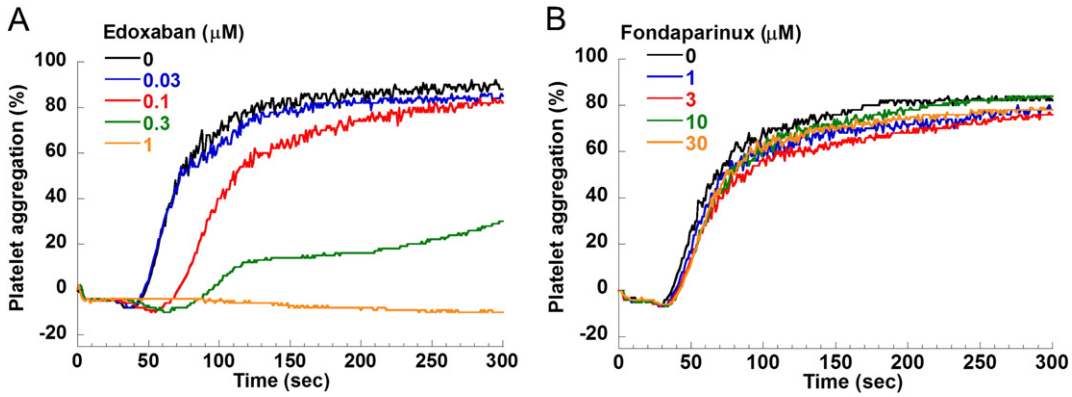

Fig. 1. The representative tissue factor (Dade Innovin)-induced platelet aggregation and effects of edoxaban and fondaparinux in human platelet-rich plasma. (A) Edoxaban and (B) fondaparinux. Human platelets were stimulated with Dade Innovin at the concentration to induce maximum platelet aggregation.

associated with thrombi, FXa is resistant to inhibition by antithrombin-dependent anticoagulants [13]. Therefore, the inhibition of clot-associated FXa by direct (antithrombin-independent) FXa inhibitors seems more effective than by indirect (antithrombin-dependent) FXa inhibitors for the prevention of thrombosis.

In our previous studies, the antithrombotic effects of a direct FXa inhibitor, edoxaban, and an antithrombin-dependent FXa inhibitor, fondaparinux, were compared in rat arterial thrombosis models [18]. Edoxaban inhibits arterial thrombosis at similar doses as in venous models. However, fondaparinux exerts an only partial antithrombotic effect at more than 60-times higher doses in arterial thrombosis models compared with venous models [18].

We raised the hypothesis that edoxaban would have more potent inhibitory effects on tissue factor-induced platelet aggregation and clot-bound FXa compared with fondaparinux. This difference might explain at least partly the reason of the partial ineffectiveness of fondaparinux in arterial thrombosis models.

2. Materials and methods

2.1. Materials

Edoxaban was synthesized by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Fondaparinux was purchased from GlaxoSmithKline K.K. (Tokyo, Japan). Dade Innovin and sodium citrate (38 mg/mL solution) were purchased from Sysmex Corporation (Kobe, Japan). HemosIL RecombiPlasTin (RecombiPlasTin) was purchased from LSI Medience Corporation (Tokyo, Japan). Human FXa was purchased from Enzyme Research Laboratories, Inc. S-2222 was purchased from Sekisui Medical Co., Ltd. (Tokyo, Japan). Human prothrombin was purchased from Sekisui Diagnostics (Lexington, MA). Human antithrombin was purchased from Hyphen BioMed (Neuville-sur-Oise, France). Enzygnost F1 + 2 monoclonal was purchased from Siemens Healthcare Diagnostics K.K. (Tokyo, Japan). Pefabloc FG (H-Gly-Pro-Arg-Pro-OH AcOH) was obtained from Pentapharm (Basel, Switzerland).

2.2. Tissue factor-induced platelet aggregation

Human platelet-rich plasma (PRP) from ten healthy volunteers both men and women were used. The study was performed in accordance with the Declaration of Helsinki and was approved by the ethics committees of the research institutes of Daiichi Sankyo Co., Ltd. Written informed consent was obtained from each participant. Blood (18 ml) was collected into a syringe containing 2 ml of 38 mg/ml sodium citrate solution. Two syringes, in total 40 ml citrated blood, was collected from each individual. PRP was separated by centrifugation at 250 $\times g$ for 6 min at room temperature. Platelet-poor plasma (PPP) was obtained from the residue by centrifugation at 1870 $\times g$ for 10 min at room temperature. Platelet numbers in PRP were adjusted to 2×10^5 platelets/μl by adding autologous PPP. Edoxaban was dissolved in 10% dimethyl sulfoxide (DMSO) in saline. Fondaparinux was diluted with saline.

Tissue factor-induced platelet aggregation was measured as reported in an earlier study [9]. PRP (240 μl) was pretreated with 10 μl of H-Gly-Pro-Arg-Pro-OH (final concentration 3 mM) for 2 min at 37 °C to prevent fibrin polymerization. Then 5 μl of edoxaban (final concentration 0.03–1 μM) or fondaparinux (final concentration 1–30 μM) was added to the plasma and incubated for 2 min at 37 °C. Platelet aggregation was induced by the addition of the mixture (15 μl of Dade Innovin or 25 μl of RecombiPlasTin) of human tissue factor and $CaCl_2$ (final concentration 7.5 mM) and measured with an optical aggregometry MCM

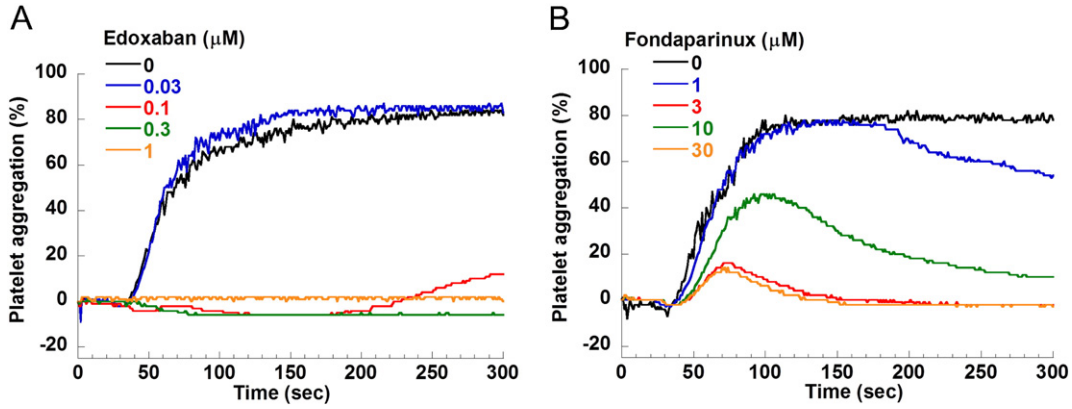

Fig. 2. The representative tissue factor (RecombiPlasTin)-induced platelet aggregation and effects of edoxaban and fondaparinux in human platelet-rich plasma. (A) Edoxaban and (B) fondaparinux. Human platelets were stimulated with RecombiPlasTin at the concentration to induce maximum platelet aggregation.