Dabigatran inhibits the activation of endothelial progenitor cells induced by thrombin

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PURPOSE

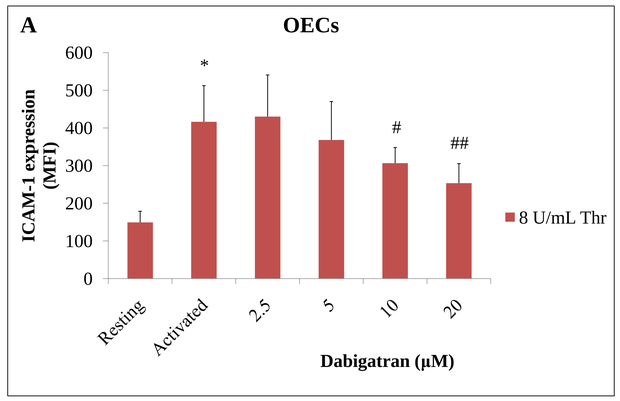
Thrombin is a coagulation serine protease, which also activates various cell types, including endothelial cells, leading to the expression of inflammatory cytokines and adhesion molecules[1]. Late-outgrowth endothelial cells (OECs) are a type of non-hematopoietic endothelial progenitor cells that contribute to endothelial regeneration and angiogenesis[2]. Dabigatran is a direct oral anticoagulant that inhibits thrombin's action and is widely used in everyday clinical practice[3]. The purpose of the present study was to investigate the effect of dabigatran on thrombin-induced activation of OECs, using the membrane expression of adhesion molecule ICAM-1 (intercellular adhesion molecule-1) as activation marker. Control experiments were conducted in mature human umbilical vein endothelial cells (HUVECs).

MATERIALS AND METHODS

$CD34^+$ cells were isolated from human cord blood mononuclear cells, using human CD34 Microbead Kit and appropriately cultured for 30 days for OEC formation. HUVECs were purchased from Lonza. Confluent OECs (passage 4) and HUVECs (passage 3) were incubated with 2.5-20 μM dabigatran (active metabolite, MedChem Express) for 10 min, before activation with 8 U/mL thrombin, for 24 h. The effect of dabigatran on ICAM-1 expression (anti-CD54-PE) was evaluated in both cell types as mean fluorescence intensity (MFI) and as % gated $CD31^+/CD54^+$ cells, using flow cytometry.

RESULTS

Thrombin induced ICAM-1 membrane expression on OECs and HUVECs by 270±47% and 494±124%, respectively, considering the MFI values and by 33±15% and 123±22%, respectively, considering the % gated cells, compared with respective untreated cells ($p<0.05$ for all comparisons, from at least 3 different experiments). Dabigatran inhibited dose-dependently thrombin-induced ICAM-1 expression on OECs, as well as on HUVECs (Figures 1 and 2). The maximum inhibition of ICAM-1 expression on OECs and HUVECs was observed at the dose of 20 μM of dabigatran and was 63±10% and 75±15%, respectively, considering the MFI values and 52±10% and 70±19%, considering the % gated cells, respectively.



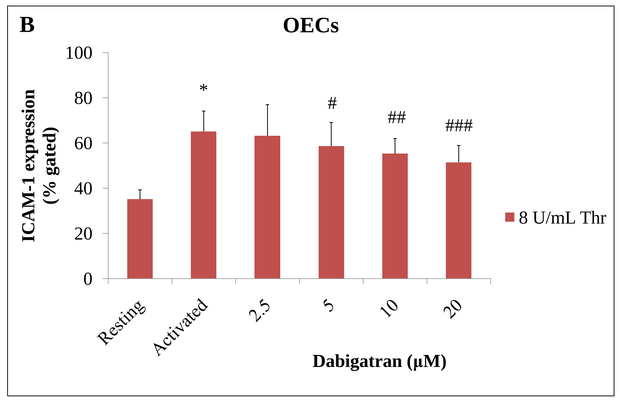
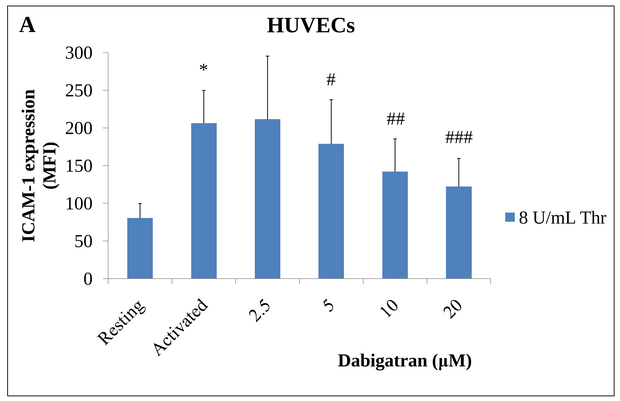


Figure 1. *The effect of dabigatran on thrombin-induced ICAM-1 expression on OECs.* OECs were incubated for 10 min with various concentrations of dabigatran ranging from 2.5-20 μM and then were activated with 8 U/mL thrombin, for 24 h. Results represent the mean±SD from at least 3 different experiments and are shown (A) as MFI values and (B) as % gated cells. (A) *p=0.012, compared to resting cells, [for the various concentrations of dabigatran: #p=0.043; ##p=0.005, compared to activated cells]. (B) *p=0.012, compared to resting cells, [for the various concentrations of dabigatran: #p=0.01; ##p=0.026; ###p=0.011, compared to activated cells].



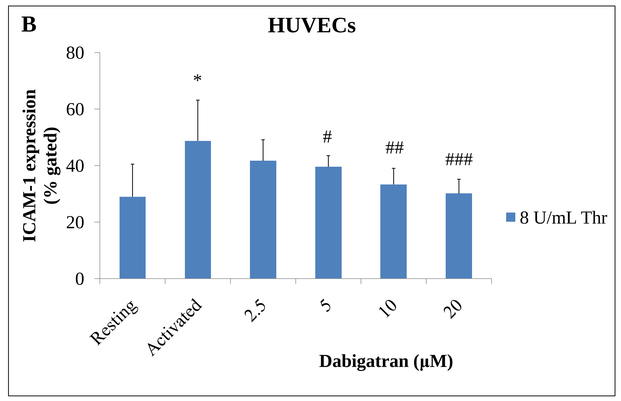


Figure 2. *The effect of dabigatran on thrombin-induced ICAM-1 expression on HUVECs.* HUVECs were incubated for 10 min with various concentrations of dabigatran ranging from 2.5-20 μM and then were activated with 8 U/mL thrombin, for 24 h. Results represent the mean±SD from at least 3 different experiments and are shown (A) as MFI values and (B) as % gated cells. (A) *p=0.001, compared to resting cells, [for the various concentrations of dabigatran: #p=0.028; ##p=0.004; ###p=0.011 compared to activated cells]. (B) *p=0.0001, compared to resting cells, [for the various concentrations of dabigatran: #p=0.007; ##p= 0.007; ###p=0.032, compared to activated cells].

CONCLUSIONS

We show for the first time that dabigatran at concentrations relevant to those existing *in vivo* after oral administration, inhibits the thrombin-induced membrane expression of the adhesion molecule ICAM-1 on OECs, a phenomenon that is also observed on mature endothelial cells. The significance of this effect regarding the pathophysiological role of OECs and mature endothelial cells at the clinical level remains to be established.

DECLARATION OF INTEREST

The authors have nothing to declare

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