Supplement Material

Materials and Methods

Sonoclot coagulation analysis

Whole blood was collected from the retro-orbital plexus and coagulation was immediately assessed using the Sonoclot analyzer and non-activated clotting test kit (Sienco). The analysis cuvettes were preheated at 37°C. Citrated whole blood (0.28 mL) was recalcified with 20 µL of calcium chloride (150 mmol/L) and analyzed. We measured 1) onset of clot formation (time in seconds to first fibrin formation) and 2) the rate of fibrin polymerization (clot rate), which is an index of fibrinogen conversion into fibrin gel.

ApoA-I infusion for DVT experiments

Native human apoA-I (Genway; 3.5 mg/kg body weight) in vehicle buffer (10 mmol/L NH_4HCO_3, pH 7.4) was infused into WT C57BL/6J, SR-BI$^{-/-}$ or eNOS$^{-/-}$ mice as a bolus intravenously via retro-orbital plexus immediately after IVC stenosis induction. Control mice received the same volume of the vehicle alone. Mice were sacrificed 6 h later and thrombi weight and length were measured.

Platelet isolation and aggregation

Whole blood was drawn from the retro-orbital venous plexus and stabilized with heparin (7.5 U/mL). Blood was centrifuged (80 x g, 10 min), platelet-rich plasma was transferred to a fresh tube, incubated with PGI_2 (1 µg/mL, 5 min, 37°C) and centrifuged at 600 x g

for 3.5 min. The platelet pellet was resuspended in Tyrode's-HEPES buffer (137 mmol/L NaCl, 2 mmol/L KCl, 12 mmol/L $NaHCO_3$, 0.3 mmol/L NaH_2PO_4, 5.5 mmol/L glucose, 5 mmol/L HEPES, 0.35% bovine serum albumin), and stored at 37°C for no longer than 1 h before use in experiments.

Platelet aggregation was evaluated using a Chrono-Log 4-channel optical aggregation system (Chrono-Log). Washed platelets ($2.5 \times 10^5/\mu L$) were incubated with apoA-I (45 μg/mL) for 5 min at 37°C, after which thrombin (Sigma, 0.1 or 1 U/mL), collagen (Nycomed, 1 or 10 μg/mL) or calcium ionophore (A23187, Sigma, 10 μmol/L), and light transmission was recorded over 10 min. The apoA-I concentration corresponds to the expected concentration of apoA-I reached in the blood of a mouse of 25 g with a blood volume of 2 mL after infusion of 3.5 mg/kg of apoA-I, assuming no losses due to distribution into tissues or degradation/excretion.

ELISA for D-dimers

Blood was drawn into 1/9 of its volume of 3.8% sodium citrate from the mouse retroorbital plexus. Plasma was obtained by centrifugation at 2300 x g and stored at -80°C until the D-dimer level was determined using an ELISA kit (Diagnostica Stago, Parsippany, NJ) according to the manufacturer's instructions.

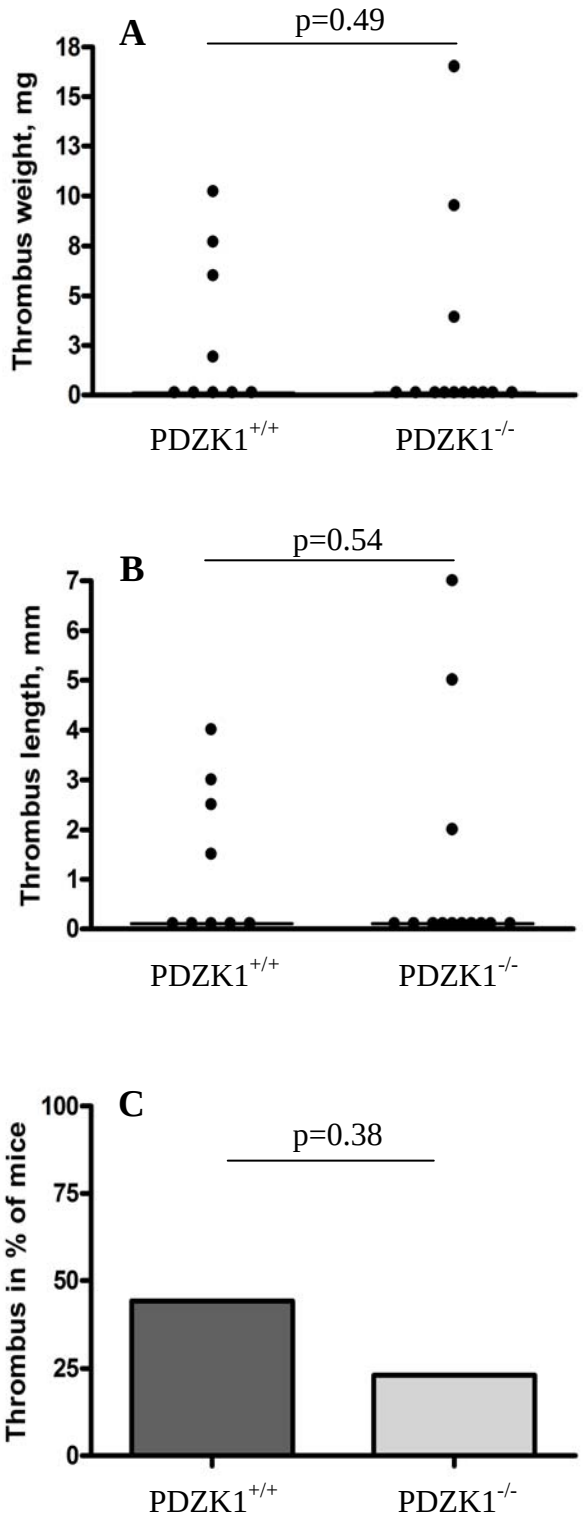
A
p=0.49
Thrombus weight, mg
18
15
13
10
8
5
3
0
PDZK1+/+
PDZK1-/-
B
p=0.54
Thrombus length, mm
7
6
5
4
3
2
1
0
PDZK1+/+
PDZK1-/-
C
p=0.38
Thrombus in % of mice
100
75
50
25
0
PDZK1+/+
PDZK1-/-

Figure I

Figure I. PDZK1 deficiency does not promote DVT. IVC stenosis surgery was performed in WT (n = 9) and PDZK1$^{-/-}$ (n = 13) mice. For both genotypes, mice were sacrificed after either 6 or 8 h (two independent experiments), thrombi excised and their weights and lengths measured. As no differences were observed between results for 6 and 8 h DVT, the results of the two experiments were combined. (A) thrombus weight in mg; (B) thrombus length in mm; (C) percent of mice that developed a thrombus (thrombi prevalence). Horizontal bars in dot plots represent median values. These results indicate that PDZK$^{-/-}$ mice do not have a prothrombotic phenotype.