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Coagulation activation during air travel is not initiated via the extrinsic pathway

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Abstract

Background: While air travel is a well-established risk factor for venous thrombosis, the underlying mechanism is still incompletely understood. We previously demonstrated coagulation activation during air travel in healthy individuals, as assessed by a rise in thrombin-antithrombin complexes. It has been suggested that venous thrombosis is initiated via the extrinsic pathway of coagulation, specifically via tissue factor-bearing microparticles. **Objective:** To determine whether coagulation activation during air travel proceeds via the extrinsic pathway. Assays that specifically detect the activated form of factor VII were used as a marker of extrinsic activation. **Methods:** We measured activity and antigen levels of FVII and FVIIa in 71 individuals before, during, and after an 8 hour flight, a movie marathon, and daily life routine. Activity levels were determined using commercially available assays. Antigen levels were determined using assays recently developed in our laboratory. **Results:** Activity levels of FVII and FVIIa were correlated with antigen levels (FVII $r = 0.373$ (95% confidence interval [CI] 0.301-0.441); FVIIa $r = 0.265$ (95% CI 0.188-0.339)). FVII activity and antigen levels did not change during or after the flight, movie marathon, or daily life routine. FVIIa activity increased after all exposures. In contrast, FVIIa antigen levels decreased during all three exposures and normalized thereafter. Activity and antigen levels of FVII and FVIIa were not different in individuals who had increased thrombin-antithrombin levels after the flight compared to those who had not. **Conclusion:** Using two distinct assays that specifically detect activated FVII, we did not find any evidence for extrinsic coagulation activation during air travel.

Introduction

Air travel has already been linked to venous thrombosis in the 1950s [1]. More recently, it has been firmly established that air travel is associated with a 2-4 fold increased risk of venous thrombosis [2,3]. The mechanisms underlying air travel-related venous thrombosis are incompletely understood but likely include immobilization and hypoxia [4-7]. However, a recent study showed that neither immobilization nor hypoxia individually affect coagulation activation, and thus a combination of factors appears to explain air travel-related thrombosis [8]. Individuals with genetic or acquired hypercoagulability appear to have a particularly increased risk of venous thrombosis after air travel [2,9]. We previously demonstrated coagulation activation after air travel in some, but not all, healthy individuals [10]. Seventeen percent of these subjects had increased thrombin-antithrombin (TAT) complexes after an 8 hour flight, but not after an 8 hour movie marathon, or an 8 hour daily life routine. It has not been established how activation of the coagulation system occurs in these subjects. Knowledge on the initiating trigger of coagulation activation after air travel may give a clue on the mechanism of air travel-related thrombosis.

It has been suggested that tissue factor-bearing microparticles are the initiating trigger in venous thrombosis in general [11-14]. We therefore hypothesized that coagulation activation after air travel proceeds via the tissue factor pathway. Here we used two distinct assays that assess plasma levels of activated (but not zymogen) factor VII as markers for extrinsic coagulation activation. As tissue factor-mediated initiation of coagulation starts with the conversion of FVII to FVIIa, we assume increased plasma levels of FVIIa to reflect TF-mediated coagulation activation. Next to a commercially available activity assay, we used an antigen assay that we recently developed which is based on a llama-derived antibody fragment. Using this latter assay, we recently demonstrated elevated levels of FVIIa to be associated with an increased mortality risk in patients with the systemic inflammatory response syndrome [15]. Additionally, we measured activity and antigen levels of factor VII.

Methods

Study design

Recruitment of volunteers and the procedures of the study were described earlier [10]. In short, 71 healthy volunteers were followed for 8 h during a flight and, as two control periods, during an 8 h movie marathon, and 8 h during daily life routine. The study included participants with the factor V Leiden mutation (n = 11), those who took oral contraceptives (n = 15), or both (n = 15), and 30 individuals with no specific risk factors

for venous thrombosis. In all three situations blood was drawn into 3.2% sodium citrate (9:1, v/v) before, during, and after the exposure. Within 15 min after collecting the blood, samples were centrifuged twice at 2500 g for 15 min at 15°C. The plasma samples were snap-frozen and stored at -80°C until use.

Plasma FVII and FVIIa levels

Factor VII activity was determined on an automated coagulation analyser (Behring Coagulation System, Siemens Healthcare Diagnostics, Marburg, Germany) with reagents and protocols from the manufacturer. Plasma FVIIa activity was measured using a commercially available kit according to manufacturer's instructions (STACLOT VIIa-rTF, Diagnostica Stago, France). From the 71 volunteers the following numbers of samples were available for analysis: flight 71-70-66, movie marathon 70-70-69, daily life routine 71-68-70, before-during-after respectively.

The plasma FVII and FVIIa antigen levels were measured using in-house developed enzyme-linked immunosorbent assays (ELISAs) as described earlier [15]. In short, plasma levels of FVII and FVIIa were determined using a semi-automated ELISA on a Tecan Freedom EVO robot (Tecan, Männedorf, Switzerland). A sheep anti-FVII antibody (Stago, Leiden, the Netherlands) or a llama-derived antibody fragment directed against FVIIa were immobilised on a 384-well plate (Thermo Fisher Scientific Inc., Waltham MA, USA). After blocking, diluted plasma samples were added to the plate and incubated. Bound FVII was detected using horseradish peroxidase (HRP) labeled sheep anti-FVII (Stago, Leiden, the Netherlands). FVIIa was detected using a chicken anti-FVII (Abcam, Cambridge, UK) antibody followed by a HRP-labeled donkey anti-chicken antibody (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA). Subsequently, a chemiluminescent substrate (SuperSignal West Pico Chemiluminescent Substrate, Thermo Fisher Scientific Inc., Waltham MA, USA) was added and after 60 min the luminescence was measured using a Spectramax reader (Molecular Devices, MDS Analytical Technologies, Sunnyvale, CA, USA). The luminescence values of the plasma samples were translated to FVII or FVIIa levels using an 8 point calibration curve of pooled normal plasma (for FVII) or recombinant FVIIa (NovoSeven, Novo Nordisk, Bagsvaerd, Denmark). From the 71 volunteers the following number of samples were available for analysis: flight 69-70-65 (FVII)/66 (FVIIa), movie marathon 70-62-71, daily life routine 71-71-71, before-during-after respectively.

Data analysis

Spearman's correlation coefficient was used to assess the correlation between the activity and antigen levels of FVII and FVIIa. The FVII and FVIIa activity and antigen levels of all individuals are presented as median plasma levels. In a first analysis, absolute changes in the variables for each individual were calculated by subtracting the pre-exposure value from the post-exposure value. Medians with 95% confidence intervals (CI) of these individual changes are presented. The change in levels of each marker for exposure to the flight, to the movie marathon, and to the daily life routine was compared with the Friedman test. In a second analysis we specifically looked at those individuals who in a previous analysis had showed coagulation activation, as evidenced by increased thrombin-antithrombin (TAT) complexes ($n = 11$), which occurred predominantly during the flight exposure [9]. These high responders for each assay were identified by using as cut-off point the 85th percentile for the absolute change in that assay during the daily life routine. For the flight situation, absolute changes for each parameter in volunteers with an activated clotting system were compared with that of volunteers without an activated clotting system by a Mann-Whitney U-test. All statistical analyses were performed with IBM SPSS Statistics, PC (release 20, IBM, New York, USA).

Results and discussion

Plasma activity and antigen levels of FVII were correlated as shown in Figure 1A ($r = 0.373$ (95% CI 0.301-0.441)). However, antigen levels of FVII of some individuals were discordant with the activity levels. Plasma activity and antigen levels of FVIIa were correlated as shown in Figure 1B ($r = 0.265$ (95% CI 0.188-0.339)). The correlation between the assays was poor at low levels of FVIIa, which may be related to the sensitivity of the antigen assay, since the low antigen levels were close to the detection limit of the assay.

Subsequently, we analysed plasma levels of FVII and FVIIa activity and antigen in samples taken of healthy volunteers before, during, and after an 8 hour flight, 8 hour movie marathon, and 8 hour daily life routine. Figure 1C shows the median FVII and FVIIa activity levels before, during, and after each exposure. Plasma FVII activity levels did not change during and after exposure to the flight, movie marathon, and daily life routine. The plasma FVIIa activity levels were higher after the flight, movie marathon, and daily life routine compared to the FVIIa levels before and during the exposures. Table 1A shows the median absolute individual changes after each exposure. The absolute individual changes were similar for all exposures. Taken together, these results do not suggest

activation of zymogen FVII during and after air travel. Since activation of coagulation via the extrinsic pathway of coagulation results in FVII to FVIIa conversion, it appears that coagulation activation after air travel does not proceed via extrinsic activation. A lack of diurnal variation of FVII has been described previously. However, the increase in VIIa activity over time is not in line with a previous study that showed FVIIa activity, measured with the same assay, to decline during the day [16].

Figure 1D shows the median FVII and FVIIa antigen levels. In line with the results of the activity assays, FVII antigen levels were equivalent throughout all three exposures. In contrast with the results of the activity assays, FVIIa antigen levels dropped during all three exposures. FVIIa antigen levels were slightly lower compared to baseline values after the flight and movie marathon, but slightly higher than baseline after the daily life routine. The reason for the clear discrepancy between the FVIIa activity and antigen tests is, at present, unclear. One explanation would be that the antigen test recognizes both 'active' FVIIa and FVIIa in complex with inhibitors. However, we have demonstrated that the assay recognizes both free FVIIa and FVIIa in complex with tissue factor, but not the FVIIa-antithrombin complex (data not shown). Although the pattern of FVIIa activity and antigen levels over time is inconsistent, the results of both assays indicate that coagulation activation during air travel does not result from activation of the extrinsic pathway.

Finally, we analysed FVII and FVIIa activity and antigen levels in those individuals who showed an appreciable increase in TAT complex levels after the flight compared with those who did not [10]. FVII and FVIIa activity and antigen levels did not differ between those individuals with an activated clotting system after the flight and those without evidence of coagulation activation (Table 1B).

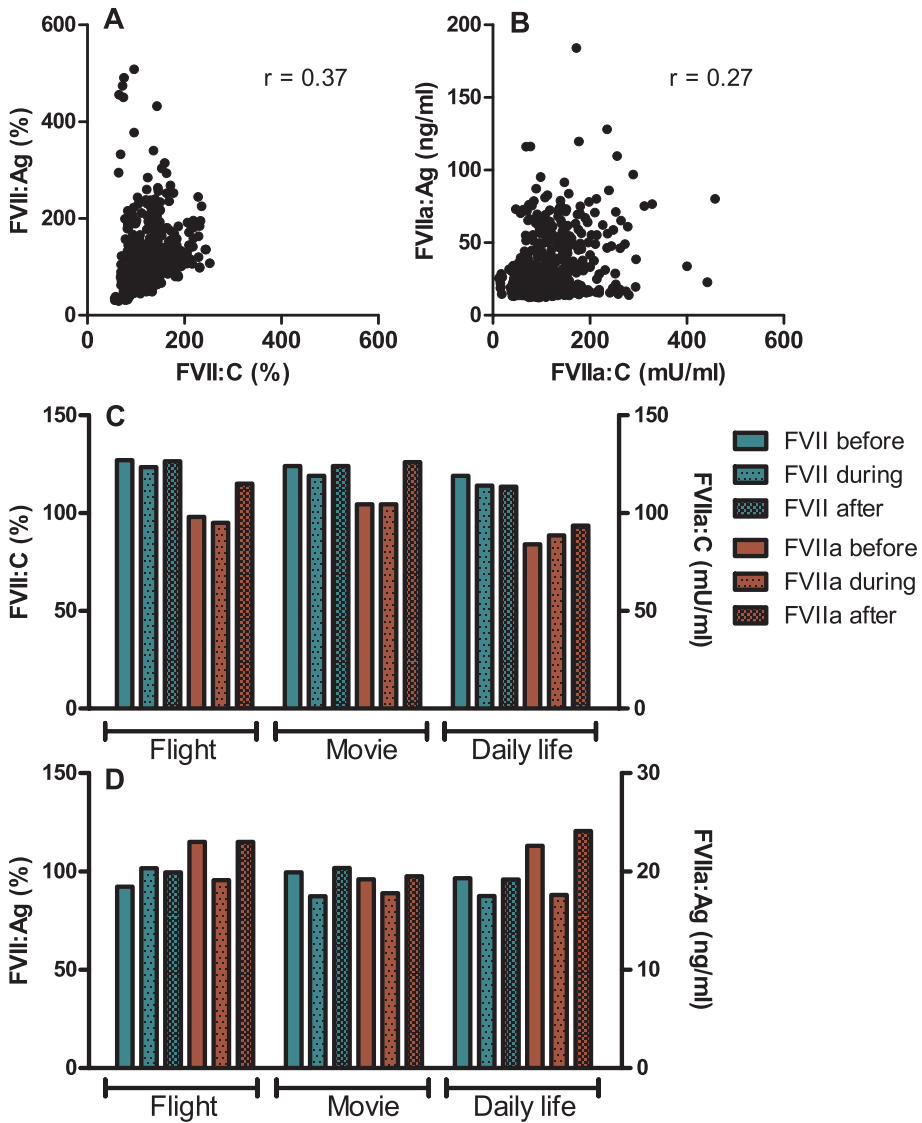


Figure 1 Correlation between plasma activity and antigen levels of FVII (A) and FVIIa (B). Plotted are the FVII and FVIIa activity and antigen levels of all individual samples in the study (71 individuals sampled at nine different occasions). Median plasma levels of FVII and FVIIa activity (C) and antigen (D) of 71 individuals that were sampled before, during, and after an 8 hour flight, movie marathon, and daily life routine. FVII:C, factor VII coagulant activity; FVII:Ag, factor VII antigen; FVIIa:C, activated factor VII coagulant activity; FVIIa:Ag, activated factor VII antigen.

Taken together, the results of this study using two distinct assays for FVII and FVIIa do not provide evidence for activation of the extrinsic pathway of coagulation during air travel. We hypothesized that air travel-related activation of coagulation would proceed via TF-bearing microparticles analogous to the proposed role of TF in development of venous thrombosis. Nevertheless, previous studies performed in the cohort described in this paper are consistent with the alternative scenario, i.e. that the intrinsic pathway is responsible for air travel-related activation of coagulation [17]. These previous studies showed higher plasma soluble P-selectin and plasminogen activator inhibitor type 1 (PAI-1) levels in those individuals with elevated TAT levels after the flight. These findings may reflect increased platelet activation in the individuals with activated coagulation after air travel. Platelet activation results in the release of polyphosphates which, among other procoagulant effects, activate the intrinsic pathway of coagulation [18].

The increased levels of P-selectin and PAI-1 may also reflect endothelial cell activation, which may initiate thrombus formation both via direct (TF-mediated) activation of coagulation or via platelet recruitment and activation. Thus, although the exact mechanisms of initiation of coagulation during air travel need to be established, the results of the present study strongly suggest that activation via the extrinsic pathway of coagulation does not play a major role.

Table 1 Median absolute individual changes in FVII and FVIIa activity and antigen levels with 95% confidence intervals (CI) after an 8 h flight, movie marathon and daily life routine in all participants (Table 1A) and after the flight in volunteers with (n = 11) and without (n = 55) an activated clotting system as defined by the change in TAT values (Table 1B).

	Flight	Movie	Daily life	P-value*
Factor VII:C (%)	4.5 (0.0 to 9.0)	1.5 (-1.0 to 7.0)	-2.0 (-5.0 to 2.0)	0.142
Factor VIIa:C (mU/ml)	16.5 (8.0 to 23.0)	16.5 (0.0 to 25.0)	8.5 (1.0 to 16.0)	0.140
Factor VII:Ag (%)	0.8 (-16.2 to 10.4)	-5.5 (-10.8 to 4.2)	-3.4 (-12.3 to 12.0)	0.895
Factor VIIa:Ag (ng/ml)	-2.5 (-5.1 to -1.1)	-0.8 (-1.9 to 1.3)	0.6 (-0.4 to 1.8)	0.015

	Volunteers with an activated clotting system	Volunteers without an activated clotting system	P-value**
Factor VII:C (%)	-4.0 (-17.0 to 23.0)	5.0 (0.0 to 10.0)	0.46
Factor VIIa:C (mU/ml)	25.0 (-33.0 to 86.0)	16.0 (8.0 to 21.0)	0.79
Factor VII:Ag (%)	0.3 (-55.8 to 47.5)	0.2 (-17.1 to 7.8)	0.81
Factor VIIa:Ag (ng/ml)	-7.0 (-41.2 to 1.0)	-2.3 (-5.1 to -0.1)	0.23

FVII:C, factor VII coagulant activity; FVII:Ag, factor VII antigen; FVIIa:C, activated factor VII coagulant activity; FVIIa:Ag, activated factor VII antigen; TAT, thrombin-antithrombin. * Friedman test, **Mann-Whitney U test.

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