The effect of β-receptor blockade on factor VIII levels and thrombin generation in patients with venous thromboembolism

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Summary
High factor VIII (FVIII) is a risk factor for venous thromboembolism (VTE). The pathomechanism by which high FVIII leads to an increased risk of VTE is unknown. Physical activity and infusion of adrenaline provoke a rise in FVIII, which can be blocked by a nonselective β-blockade. We tested the hypothesis that in patients with a VTE β-blockade decreases FVIII and inhibits coagulation activation.

17 male patients with high FVIII (> 170 IU/dL, n = 7) or low FVIII (<150 IU/dL, n = 10) and a history of VTE received 40 mg of propranolol thrice daily for 14 days. FVIII and vasopressin levels were measured before and during propranolol intake and 28 days thereafter. At the same time points, haemostatic system activation was investigated by measuring prothrombin fragment f1.2 (f1.2) and thrombin antithrombin complexes (TAT) in venous blood and in blood emerging from a skin incision (shed blood).

The mean FVIII level before propranolol was 192 IU/dL and 115 IU/dL in patients with high and low FVIII, respectively. During and 28 days after propranolol, no significant change in FVIII was seen in both groups. Changes in f1.2 and TAT were not detectable in either venous blood or in shed blood.

β-receptor blockade did not lower FVIII or inhibit haemostatic system activation in patients with VTE and persistently high FVIII. Administration of propranolol cannot be recommended as secondary thromboprophylaxis in patients with high FVIII.

Keywords
Venous thrombosis, factor VIII, β-receptor blockade, thrombin generation

Introduction
A high plasma concentration of coagulation factor VIII (FVIII) is an important risk factor for venous thromboembolism (VTE) (1-4). In the Leiden Thrombophilia study for example, FVIII levels above 1500 IU/L conferred an almost 5-fold risk for a first episode of deep-vein thrombosis (1). Most importantly, high FVIII is also associated with a very high risk of recurrent venous thromboembolism (4, 5). High factor VIII levels persist over time (3, 5) and are not attributable to an acute phase reaction (2-6). The pathomechanism(s) by which high levels of FVIII lead to an increased risk of venous thrombosis are still unknown.
Recurrence is a serious complication of VTE with a case-fatality rate of approximately 5% (7). Recurrent VTE can be effectively prevented by oral anticoagulant therapy and patients with a high risk of recurrent venous thromboembolism are therefore candidates for extended secondary thromboprophylaxis. The price for effective thrombosis prevention is severe, sometimes even resulting in fatal bleeding (8). With regard to patients with high FVIII, further clinical trials investigating the effect of long-term anticoagulation are required.

Both physical activity and infusion of adrenalin or vasopressin enhance FVIII and von Willebrand factor levels (9, 10). The effect of adrenalin on FVIII levels can be blunted by nonselective \( \beta \)-blockade (9, 11, 12). Recently, Kraaiaijen et al. treated patients with venous thrombosis and FVIII levels above 175 IU/dL with propranolol, a non-selective \( \beta \)-receptor blocker, and found a 25% reduction in FVIII with a mean decrease of 50 IU/dL (13). The decrease in FVIII ran in parallel with a reduction in the vasopressin plasma levels. Propranolol was ineffective in terms of lowering FVIII among thrombosis patients with lower FVIII levels and in healthy volunteers.

This study was conducted to investigate whether patients with a history of venous thrombosis and high FVIII levels, who were administered a non-selective \( \beta \)-receptor blocker, showed lower FVIII and inhibited haemostatic system activation in venous blood and in blood obtained directly from microcirculation when collected from a local vascular injury site made to determine bleeding time (shed blood).

**Methods**

**Patients**

The study group consisted of 17 male patients (median age 52 years, range 20 to 69 years) with a history of objectively documented VTE who had stopped taking oral anticoagulation at least 3 months prior to study commencement. These patients had taken part in a large prospective cohort study (Austrian Study on Recurrent Venous Thromboembolism – AUREC) which was aimed at identifying patients with a high risk of recurrent VTE (4). VTE had occurred spontaneously in 13 patients and in 4 patients following to surgery or trauma. None of the patients had cancer, a lupus anticoagulant, or protein C-, protein S-, or antithrombin- deficiency. Two patients were heterozygous for the Factor V Leiden mutation and 2 patients were heterozygous for the G20210A mutation in the prothrombin gene. At baseline, the FVIII plasma level was >170 IU/dL in 7 patients and was <150 IU/dL in 10 patients. All study subjects had normal values of C reactive protein and fibrinogen at the time of FVIII measurement. None of the patients had taken \( \beta \)-blocker for at least 3 months prior to the study. The study was approved by the Ethics Committee of the Vienna University School of Medicine. Written informed consent was obtained from all patients before inclusion in the study.

**Study design and blood sampling**

Before entry into the study a thorough history, a physical examination, measurement of the blood pressure, ECG and various blood tests were performed by an independent cardiologist.

The study patients received 40 mg of propranolol orally thrice daily for a total period of 14 days. At day 1 (before the first dose of propranolol = baseline), day 14 (2 h after the last dose of propranolol) and day 42 (28 days after the last dose of propranolol), venous and shed blood was collected as described below.

Venous blood was drawn by puncturing an antecubital vein without the use of a turniquet into ice-cooled plastic tubes containing 1/10 volumes of 3.8% sodium citrate, EDTA or a 1/20 volumes of a stop solution consisting of 100 mM EDTA, 30 \( \mu \)mol/L indomethacin, 3.8% sodium citrate, 1000 U/ml sodium heparin and 1000 U/mL aprotinin, respectively. After mixing, the tubes were immediately centrifuged at 3500 g for 10 min. The supernatant was removed and stored at -80°C.

After venous blood sampling, bleeding time incisions were performed as described by Mielke et al. (14). Briefly, a sphygmonanometer cuff was placed at the upper arm and inflated to 40 mm Hg. Two incisions, 5 mm long and 1 mm deep, were placed on the lateral aspect of the forearm parallel to the ante-cubital crease by the use of a disposable standard device (Simplate II\textsuperscript{®}, Organon Teknika Corp, NC, USA). Over a period of 4 min the blood was collected in 20 sec intervals directly from the edge of the incision and was transferred immediately into ice-cooled plastic tubes containing 100 \( \mu \)L of the stop solution described above. After mixing, the tubes were centrifuged at 12000 g for 2 min. The supernatants were removed and stored at -80°C. The procedure was carried out by the same investigator each time.

**Assays**

Both in venous blood and in shed blood, f1.2 and TAT were measured using commercially available assay kits based on ELISA technique [Enzymnost F1+2\textsuperscript{®} (Behringwerke AG, Germany) and Enzygnost TAT\textsuperscript{®} (Behringwerke AG, Germany)]. FVIII was measured by a one stage clotting assay with the use of FVIII deficient plasma obtained from Immuno Baxter (Baxter Healthcare, Vienna, Austria) and a fully automated coagulation analyzer (CA 6000, Sysmex, Kobe, Japan). Determination of vasopressin levels was carried out using a commercially available assay kit [Vasopressin 125I RIA Kit\textsuperscript{®} (DiaSorin, Minnesota, USA)].

**Data analysis**

Values are presented as mean ± SD. The Wilcoxon matched pairs test and the Mann-Whitney U test were used for compari-
son between the data sets within one group and between the two groups, respectively.

**Results**

No adverse events were encountered in any of the patients during or after administration of propranolol.

**Venous blood**

At baseline (day 1), the mean FVIII level was 115 ± 18 IU/dL in patients with low FVIII and 192 ± 24 IU/dL in patients with high FVIII. Levels of f1.2 and TAT were higher among patients with high FVIII as compared with the patients with low FVIII (Table 1).

At day 14, i.e. 2 weeks after propranolol treatment, a significant change in FVIII was not seen in either of the two patient groups [low FVIII group: 113 ± 19 IU/dL; high FVIII group: 198 ± 36 IU/dL (Fig. 1)]. Similarly, no significant difference in FVIII was observed for either groups 4 weeks after discontinuation of propranolol (= day 42) as compared with baseline [low FVIII group: 128 ± 24 IU/dL; high FVIII group: 195 ± 38 IU/dL (Fig. 1)]. At days 14 and 42, levels of f1.2 and TAT were higher among patients with high FVIII than among those with lower FVIII. No significant alterations in the f1.2 and TAT plasma concentrations were detectable at days 14 and 42 within the two patient groups as compared with the corresponding baseline values (Table 1).

Levels of vasopressin were below the detection limit of the assay system (1.0 pg/ml) in all patients at all times (data not shown).

**Shed blood**

At baseline and at days 14 and 42, f1.2 and TAT values in shed blood were higher among patients with high FVIII as compared with those with low FVIII levels (Table 1). Similar to venous blood, the levels of f1.2 and TAT in shed blood at days 14 and 42 did not significantly differ from the corresponding baseline values within the two patient groups (Table 1).

**Table 1:** Levels of activation markers in venous and shed blood obtained from patients with high and low FVIII, before (Baseline), during (Day 14) and 4 weeks after (Day 42) propranolol.

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<tr>
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<th>Patients with high FVIII</th>
<th>Patients with low FVIII</th>
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<tr>
<td></td>
<td>venous blood</td>
<td>shed blood</td>
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<tr>
<td>Baseline</td>
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<tr>
<td>f1.2 (nmol/l)</td>
<td>0.89 ± 0.14</td>
<td>32.3 ± 7.4</td>
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<td>TAT (µg/l)</td>
<td>2.26 ± 1.33</td>
<td>1208 ± 341</td>
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<td>Day 14</td>
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<tr>
<td>f1.2 (nmol/l)</td>
<td>1.02 ± 0.22</td>
<td>36.9 ± 8.1</td>
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<tr>
<td>TAT (µg/l)</td>
<td>1.63 ± 0.34</td>
<td>1381 ± 416</td>
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<tr>
<td>Day 42</td>
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<tr>
<td>f1.2 (nmol/l)</td>
<td>0.94 ± 0.23</td>
<td>30.2 ± 5.9</td>
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<tr>
<td>TAT (µg/l)</td>
<td>2.1 ± 0.68</td>
<td>1049 ± 284</td>
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* p-value for comparison between venous and shed blood, respectively

**Figure 1:** Levels of FVIII (means ± SD) in patients with high FVIII (FVIII >170 IU/dL; depicted as triangles) and in patients with low FVIII (FVIII<150 IU/dL; depicted as squares) before, 2 weeks after propranolol treatment (40mg thrice daily) and after a wash out period of 4 weeks.
Discussion

Patients with elevated plasma levels of FVIII are at a very high risk of recurrent venous thromboembolism. Besides extended oral anticoagulation, a promising therapeutic concept for this patient group with regard to secondary thromboprophylaxis might be the reduction of FVIII levels by non-selective β-blockade. To test this hypothesis, we measured FVIII and haemostatic system activation in thrombosis patients with high and low FVIII levels before, during and after treatment with propranolol, a non-selective β-receptor blocker.

The principal finding of our study was that propranolol had no effect on FVIII plasma levels. Most importantly, among patients with FVIII above 170 IU/dL at baseline, an alteration in the FVIII levels was not seen at the end of a 2-week course of propranolol or after a 4-week wash-out period. Even though we can not exclude a small effect of propranolol on FVIII levels due to the rather low number of patients investigated in our study, an important effect is unlikely. In keeping with this finding, vasopressin levels were below the detection limit of our assay system at all time points both in patients with high and low FVIII. The conclusion of our findings is that persistently elevated FVIII (i.e. not attributable to acute phase reaction or adrenergic stimulation) is – at least in our patient population – not related to a higher baseline activation of the adrenergic system. Consequently, our data do not support the lowering of FVIII levels by non-selective β-receptor blockade.

Heightened haemostatic system activation as reflected by increased levels of coagulation activation markers occurs in several subgroups of thrombosis patients. Approximately one third of individuals with protein C- or protein S- deficiency have f1.2 levels greater than the upper limit of control (15, 16). Similarly, we and others found significantly higher plasma concentrations of molecular markers of thrombosis among patients with resistance to activated protein C or hyperhomocysteinemia than among normal controls (17-20). Recently, O'Donnell et al. reported elevated TAT and f1.2 levels in the vast majority of thrombosis patients with FVIII higher than 150 IU/dL (21). In accordance with these findings, haemostatic system activation as reflected by f1.2 and TAT plasma concentrations at baseline, day 14 and day 42, was more pronounced in our patients with high FVIII (>170 IU/dL) as compared with those with lower levels of FVIII.

We have recently developed a technique that allows investigation of the mechanisms leading to plug formation and that closely reflect in vivo conditions. The method consists of a standardized injury of the microvasculature (skin template bleeding time) and subsequent measurement of coagulation specific indices in blood emerging from this local injury site (shed blood). This method has been shown to be very sensitive in detecting changes in haemostatic system activation during anticoagulation with low-molecular weight heparin or hirudin (22, 23). In the present study, levels of haemostatic system activation markers were much higher in shed blood obtained from individuals with FVIII levels above 170 IU/dL than in blood from the microcirculation of patients with lower FVIII values. At none of the time points, however, did the differences reach the level of statistical significance because of the limited number of individuals. Nevertheless, our findings of higher levels of thrombin generation markers both in venous blood and in shed blood strongly argue for the presence of a biochemically detectable hypercoagulable state in patients with a history of venous thrombosis and high FVIII levels.

When f1.2 and TAT were measured in venous blood and in shed blood at day 14 (after 2 weeks of propranolol treatment) and day 42 (4 weeks after propranolol treatment), no significant differences were found compared with the corresponding marker levels at baseline. Thus, an inhibiting effect of β-blockade on haemostatic system activation was not detectable.

In conclusion, our data do not support the concept of a persistent activation of the adrenergic system in patients with high FVIII or a history of venous thrombosis. Propranolol did not have an effect on either FVIII or on haemostatic system activation and can therefore not be recommended for secondary thromboprophylaxis in this subset of thrombosis patients.

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