

ORIGINAL ARTICLE

Concomitant infusion of low doses of rFVIIa and FEIBA in haemophilia patients with inhibitors

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Summary. Patients with severe haemophilia A and an inhibitor may become refractory to FEIBA and/or recombinant factor VIIa (rFVIIa). Sequential therapy with both products has been reported in such patients. In this pilot study, we examined the safety and efficacy of combined rFVIIa and FEIBA therapy in patients with haemophilia A and inhibitors during bleeding episodes. We also tried to evaluate whether thrombin generation (TG), by various mixtures of these agents, can serve as a guide for tailoring therapy. TG was measured in plasma taken from eight haemophilia A patients. Increasing concentrations of rFVIIa, FEIBA or both were added *ex vivo* to the plasmas, and TG was induced by recalcification. Since low concentrations of rFVIIa and FEIBA had either an additive or a synergistic effect in all patients, the lowest combination, yielding TG comparable or

lower than TG achieved with either FEIBA 100 U kg⁻¹ or rFVIIa 160 µg kg⁻¹ alone, was selected for the treatment of bleeding episodes. Five patients with a high titre of an inhibitor (8–1300 BU), including one previously refractory to infusions of rFVIIa at doses up to 400 µg kg⁻¹ X4 daily, were treated with combinations of 30–70 µg kg⁻¹ rFVIIa and 20–30 U kg⁻¹ FEIBA during a total number of 400 bleeding episodes with excellent haemostatic effect. No adverse events and no DIC were observed following these infusions. Concomitant infusion of low-dose rFVIIa and low-dose FEIBA, seems to be safe, efficacious and economical in patients refractory to rFVIIa and probably other haemophilia A patients with an inhibitor.

Keywords: FEIBA, haemophilia, inhibitors, rFVIIa

Introduction

Patients with haemophilia A and B who develop inhibitors and become refractory to replacement therapy with FVIII or IX, respectively can be treated effectively by activated prothrombin complex concentrates (APCC, e.g. FEIBA) or by recombinant factor VIIa (rFVIIa) [1–3]. When used at doses of 50–100 U kg⁻¹, APCC is effective for achieving haemostasis in 50–65% of patients with inhibitors treated in controlled trials [4–6], whereas standard

rFVIIa doses of 90 µg kg⁻¹ yield effective haemostasis in about 90% of patients treated by two to three repeated injections [7]. Recently randomized controlled trials demonstrated similar efficacy of single high-dose rFVIIa (270 µg kg⁻¹) as compared to standard regimen [6,8,9], nevertheless, some patients with inhibitors may develop refractoriness to these products [10]. For such patients, therapy with combination of standard doses of rFVIIa and FEIBA by sequential administration have been recently suggested [11]. Moreover, our group focused on the possibility and safety of coadministration of low-doses rFVIIa with FEIBA [12].

Laboratory assessment of the efficacy of bypassing agents is still a challenge. Thrombin generation (TG) was found to be a sensitive method for assessing the impact of various coagulation concentrates, including rFVIIa and FEIBA in haemophilic blood, and may enables the monitoring of pharmacodynamic

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and pharmacokinetic properties of bypassing therapies [13–15]. However, no good correlation to clinical response was found, possibly due to large inter and intra patient variability.

The impetus for the present report was a haemophilia A patient (termed as Patient 1) with high-titre inhibitor (1300 BU) who had life threatening haematuria that was resistant to repeated doses of $400 \mu\text{g kg}^{-1}$ rFVIIa, given at 2–3 h intervals up to a cumulative dose of $1200 \mu\text{g kg}^{-1}$ over 6–9 h. TG tested *in vitro* in the patient's plasma was consistent with low response to high concentrations of rFVIIa, demonstrating the patient's resistance to treatment.

In a desperate attempt to stop the uncontrolled bleeding, concomitant combined therapy (CCT) of low-doses rFVIIa and FEIBA was infused and resulted in arrest of bleeding within minutes. Following this case a study was designed aiming to evaluate the potential safety and efficacy of coadministration of low-dose rFVIIa and FEIBA in haemophilia A patients with inhibitors resistant and even non-resistant to rFVIIa/FEIBA alone, by tailoring the combination dose according to *ex vitro* TG tests.

Patients and methods

Patients

Patients with severe haemophilia A and inhibitors were eligible for the study if they were previously treated by either FEIBA or rFVIIa and gave their informed consent. Exclusion criteria were: age < 16 years or >60 years old, preexisting coagulopathy other than haemophilia, previous allergic reaction to any of the products, previous thromboembolism, presence of comorbid cardiovascular risk factors. The study was approved by the ethical committee in concordance with the declaration of Helsinki.

Reagents

rFVIIa was purchased from NovoNordisk, Copenhagen, Denmark. FEIBA was purchased from Baxter, Vienna, Austria. FEIBA and rFVIIa were prepared according to manufacturer's instructions. Synthetic phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were obtained from Avanti Polar Lipids, Alabaster, AL, USA. The thrombin-specific fluorogenic substrate Z-GGR-AMC was obtained from Bachem, Bubendorf, Switzerland. HEPES/bovine serum albumin (BSA) buffer containing 20 mM HEPES, 140 mM NaCl pH 7.3 and 5 mg mL^{-1} BSA (Sigma, St Louis, MO, USA) was used as a reaction buffer. HEPES buffer pH 7.3

containing 20 mM HEPES with 60 mg mL^{-1} BSA was used for diluting the fluorogenic substrate.

Processing of blood samples

Blood was drawn in 0.109 M buffered citrate from patients with severe haemophilia A. Platelet-poor plasma (PPP) was obtained at room temperature by centrifugation of blood at 2000 g for 10 min followed by centrifugation at 14 000 g for 5 min. Platelet-rich plasma (PRP) was prepared by centrifugation of blood at 130 g for 10 min and adjustment of the platelet count to $150\,000 \mu\text{L}^{-1}$ with autologous PPP.

Thrombin generation

TG was found to be a sensitive method for assessing bypassing agents activity in haemophilia A patients' plasma [15–17]. TG was measured in PPP or PRP of severe haemophilia A or normal controls with a fluorometric assay (Fluoroskan Ascent; Labsystem, Helsinki, Finland) as previously described [17]. Briefly, 80 μL plasma, were placed in microtitre plates (Greiner Bio-1, Frickenhausen, Germany) for fluorescence measurements. For TG measurements in PPP 20 μL HEPES/BSA containing a mixture of PS:PE:PC at a ratio of 1:1:1.25 was added with or without rFVIIa or FEIBA or both, whereas for TG measurements in PRP no exogenous phospholipids were added, and platelet counts were adjusted to $150\,000 \mu\text{L}^{-1}$ with autologous PPP. The reaction was initiated by adding 20 μL HEPES/BSA containing 100 mM CaCl_2 and 5 mM fluorogenic substrate Z-GGR-AMC. The choice to induce TG by recalcification only, without added tissue factor or without contact factors inhibition was made since according to previous experience of our group these conditions allow maximal sensitivity for assessment of bypass agents' action [15,17]. The rationale to use TG assays and not refer to classical APTT, induced with PPP and additional phospholipids similar to our PPP tests, stems from the following: APTT measures time to clot formation, whereas the TG reflects the net effect of clot generation as manifested by lag time (correlating with clotting time), time to peak, endogenous thrombin potential (ETP) and height of thrombin peak that give additional information. *Ex vivo* TG tests were performed with no addition of tissue factor and with addition of exogenous phospholipids or endogenous platelets. We intended to mimic haemostatic conditions with no trigger for coagulation, and thus to ensure that no thrombosis will occur when combined therapy is applied. Since

the system used showed high variability, all assays were compared to baseline values of the patients' plasma (in the absence of rFVIIa or FEIBA), and were carried together with the same batch of preparation. Plots of TG, i.e. lag time, ETP and peak height were displayed by a COMPUTER PROGRAM attached to the fluorometer, and all parameters were measured for all patients. From our previous studies [15], it has been obvious that both FEIBA and rFVIIa may shorten the lag time and time to peak and induce higher ETP and peak height with the latter two being more sensitive to FEIBA concentrations. Thus, among TG parameters we chose to elaborate upon peak height and ETP, as representative markers for potential TG induced by CCT.

Treatment episodes and follow-up

In order to reduce the risk for potential thromboembolic complications, we set caution limitation for the dosing of combined therapy. We defined TG parameters induced by high-therapeutic concentrations of each drug alone as the highest TG levels allowed and confirmed that TG induced by combined drug doses chosen for clinical therapy of bleeding episodes would not exceed it. The combination chosen was not the most efficient, but rather the one we presumed to be safe (not exceeding the high concentrations that may be used for each drug alone).

Patients were treated during bleeding episodes with combinations of 30–70 $\mu\text{g kg}^{-1}$ rFVIIa injected as IV bolus and immediately followed by a bolus of 20–30 U kg^{-1} FEIBA. For each patient, samples were obtained to evaluate activation of coagulation markers during one of the bleeding episodes or more: pre and post-treatment platelet counts, fibrinogen levels, D-dimers, Thrombin antithrombin (TAT) complexes and F1 + 2. Patients were clinically followed after each treatment to exclude adverse effects or any complications. Following successful therapy at the clinic, home-treatment for further bleeding episodes was allowed as an option for our patients.

Efficacy assessment of haemostatic therapy in home-treated haemophilia patients is troublesome. Naturally, joint mobility of haemophilia patients is not expected to resume within the first hours following acute haemarthrosis. In addition, pain relief is not always expected to be concurrent with improvement in mobility in patients with haemophilia. Nevertheless, a combined evaluation of pain and mobility at a given time-point, using standard scoring systems, has not been validated so far [6,8,9]. Despite the limitations of substantial risk of bias by

the patient as well as difficulties in judging the influence of arthropathy/synovitis in the clinical response, efficacy assessment in this study was based upon patient's subjective definitions for haemostasis, defining treatment response as 'poor/inadequate' if it required further rescue therapy following CCT, or 'good/sufficient' once it responded to single dose of CCT. Follow-up by phone calls discussing therapy and response with caregiver was conducted until 24 h after therapy administration.

Results

Five adult patients with severe haemophilia A and inhibitors (8–1300 BU), aged 17–60 years were treated per protocol and followed by the Israeli National Hemophilia Center from January 2005 to August 2007. Plasma taken from eight patients with severe haemophilia A was tested for *ex vivo* TG parameters measurements.

The first patient (#1) failed to respond to standard doses of either rFVIIa or FEIBA in past episodes of haemarthroses or muscle bleeding. The patient suffered uncontrolled life threatening bleeding resistant up to four doses of 400 $\mu\text{g kg}^{-1}$ rFVIIa given at 2 h intervals. Previous laboratory studies of this patient revealed normal levels of plasma factors II, VII, V and X, normal fibrinogen levels and no evidence of impaired platelet function. Since no abnormality in the levels of coagulation factor (beside FVIII) was found to the use of non-activated prothrombin

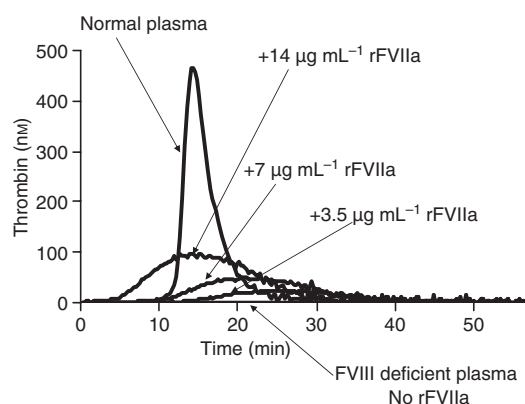


Fig. 1. Thrombin generation response induced *in vitro* in patients' 1 PPP in the absence and in the presence of increasing concentrations of recombinant factor VIIa, in comparison to TG induced in normal plasma. Thrombin generation was induced by recalcification in normal PPP in the presence of phospholipids (4 μM), or in Patients' 1 plasma in the presence of phospholipids and increasing concentrations of recombinant factor VIIa. Numbers next to the plots denote final concentrations ($\mu\text{g mL}^{-1}$) of recombinant factor VIIa.

Table 1. The synergistic effect of increasing concentrations of rFVIIa and FEIBA on ETP values (nM min^{-1}) in PRP of a haemophilia A patient with a high-titre FVIII inhibitor (Patient 1).

rFVIIa ($\mu\text{g mL}^{-1}$)	FEIBA (U mL^{-1})				
	0	0.2	0.4	0.8	1.6
0	0	162	431	651	1738
0.875	0	310	491	817	
1.75	47	611	955	1166	
3.5	292	543	1080	1321	
7	317				

complex concentrate (PCC), was ruled out. The resistance of Patient 1 to the therapy with increasing concentrations of rFVIIa, was correlated with observed *in vitro* TG response in patients' plasma (Fig. 1). TG was induced by recalcification in the absence of tissue factor and in the presence of phospholipids. As indicated from Fig. 1 no TG can be detected in FVIII deficient plasma in the absence of added rFVIIa (flat curve). Addition of rFVIIa up to very high concentrations ($14 \mu\text{g mL}^{-1}$ compatible with infusion of $600 \mu\text{g kg}^{-1}$) induced TG that was still much lower as compared to normal plasma. The poor ability of rFVIIa to correct Patient 1 TG was also demonstrated when tested in PRP as indicated from the ETP levels demonstrated in Table 1. It can be seen from the shaded boxes that rFVIIa alone induced low levels of ETP even when added to the plasma at high concentrations. High concentrations of FEIBA alone yielded pronounced ETP levels. The addition of both FEIBA and rFVIIa, in low concentrations resulted in an additive or even synergistic increase of ETP. The same phenomena of synergistic ETP increase induced by low dose of combination of both drugs, was demonstrated also when measured in PPP, as shown by Table 2 (Patient 2). In order to explore the hypothesis that rFVIIa and FEIBA has a combined effect on TG in FVIII deficient plasma, we measured the height of the thrombin peak in the presence of rFVIIa, FEIBA or both using the same type of experiments. Figure 2 demonstrates that low

Table 2. The synergistic effect of increasing concentrations of rFVIIa and FEIBA on ETP values (nM min^{-1}) in PPP of a haemophilia A patient with a high-titre FVIII inhibitor (Patient 2).

rFVIIa ($\mu\text{g mL}^{-1}$)	FEIBA (U mL^{-1})				
	0	0.2	0.4	0.8	1.6
0	0	0	0	218	502
0.875	0	0	370	453	
1.75	0	312	395	461	
3.5	327	449	484	669	
7	463				

concentrations of rFVIIa amplified the effect of FEIBA on peak height and increasing concentrations of FEIBA amplified the effect of rFVIIa on peak height.

The synergistic *in vitro* effect of low concentrations of rFVIIa and FEIBA on TG parameters was found in all eight patients' plasmas (data not shown). TG assays performed following *in vivo* administration of CCT confirmed the increase of patients' ETP and peak height as compared to baseline TG, when tested 30, 60 and 120 min following therapy during acute bleeding episodes.

Consequently, the patients were treated concomitantly by low-dose rFVIIa and FEIBA per protocol. Since, Patient 1 refused to be treated by high-therapeutic doses of FEIBA (yielding better haemostasis as compared to rFVIIa according to TG assays), combination of rFVIIa $45 \mu\text{g kg}^{-1}$ and FEIBA 25 U kg^{-1} , not exceeding ETP achieved by higher concentrations of each drug alone, was applied for therapy of further bleeding episodes in this patient. Nevertheless, relying upon TG assayed for this patient, the possibility that his haemostatic response was affected by addition of FEIBA alone can not be excluded.

A total of over 400 bleeding episodes were treated, of which most were in Patient 1, and most were applied as home therapy. Two other patients, successfully treated in our centre, continued applying the same protocol for home therapy of bleeding episodes.

The bleeding episodes consisted of haemarthroses (mainly target joint bleeds: over 300 haemarthroses of Patient 1, 10 target joint haemarthroses in patient 2, 30 and 40 episodes in Patients 3 and 4, respectively), muscle bleeds (Patient 1, about 20 occasions) and recurrent haematuria (Patient 1 only). One of our patients (#2) was treated by CCT for papilotomy and gallbladder stent insertion performed for obstructed bileduct, causing ascending cholangitis. This 60-year-old patient has been treated with doses of concomitant combined rFVIIa ($60 \mu\text{g kg}^{-1}$) and FEIBA (30 U kg^{-1}) every 6 h followed by similar doses three times and thereafter two times daily, for a total of 72 h after the procedure. Neither bleeding nor thrombosis or any other adverse event complicated the course of this patient.

The efficacy of CCT was reported by the first patient as 'remarkably better and more efficient' than when he used 'mega' doses of $300 \mu\text{g kg}^{-1}$ of rFVIIa [18] or therapeutic doses of FEIBA. There have been no adverse events. Patient 1 reported effective haemostasis following single dose CCT in 75% of bleeding episodes, whereas for other patients

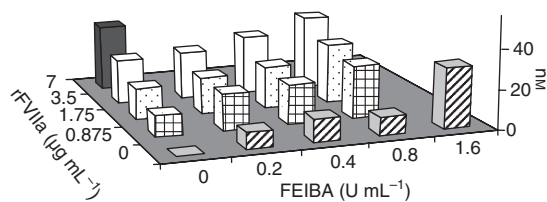


Fig. 2. The synergistic effect of increasing concentrations of rFVIIa and FEIBA on the values of the height of TG peak (nm) in PPP of a haemophilia A patient with an inhibitor to FVIII (Patient 2). The values for rFVIIa alone and FEIBA alone are shown in the shaded boxes.

treatment for haemarthroses with one dose of CCT was considered effective, if initiated early after onset of bleeding (1–2 h). Failure to stop bleeding was noted in some target joint bleeds (ankle, Patient 1), large haematomas of ileopsoas and paravertebral muscles (Patient 1) and in all cases of protracted haematuria (Patient 1) - the latter resulted in administration of further doses of CCT (to a total of 2–3 doses daily, administered q6 h) in order to achieve and maintain haemostasis. For Patients #2 and #3, the combined low-dose therapy had an excellent efficacy although they had responded to previous therapeutic doses of either rFVIIa (at standard doses or single higher doses) or FEIBA (standard doses). Patient 4 reported use of subsequent CCT doses (a total of two to three doses every 6 h) for home therapy of target joint or larger joint (e.g. shoulder) bleeding, when treatment initiation was delayed to 4 h or more postsymptoms' initiation.

No decrease in platelet counts or fibrinogen levels were documented following CCT and no clinical evidence for activation of coagulation was observed. Laboratory assays following administration of CCT disclosed a mild increase of D-dimers level only in the patient who underwent stent insertion for ascending cholangitis. In three out of five patients

tested TAT and F1 + 2 levels increased at 0.5 and 2 h post-CCT and returned to normal range within 4–6 h in all but 1 case (Table 3). Neither clinical thrombosis nor any other serious adverse event was observed in the fifth patient.

Discussion

Some haemophilia patients with inhibitors may become refractory to therapy with either rFVIIa or APCC. Management of such patients is difficult, being associated with higher morbidity and potential mortality, as well as very high costs.

Combination therapy with both drugs was suggested as a 'last resort' treatment for bleeding episodes in such patients.

Combination therapy with sequential administration of standard doses of APCC and rFVIIa, has been recently reported to be safe and effective in 35 refractory bleeding episodes in four young haemophilia patients [19]. In a retrospective review of surgical interventions in a cohort of haemophilia A patients with inhibitors, combined therapy with both agents, given at standard therapeutic doses either sequentially or concomitantly (e.g. bolus doses of FEIBA applied during continuous infusion of rFVIIa) was also described [20]. The use of sequential therapy in patients suffering refractory bleeding episodes was recently recommended within the guidelines for optimal care of high-titre inhibitor patients, published by an international panel of physicians [21].

Studies by our group have shown that rFVIIa and FEIBA yield an additive or synergistic TG increase when tested together *in vitro* [15]. This finding is in concordance with previous reports, including *in vivo* and cell-based models, elaborating on the contribution of coagulation factors, phospholipids and platelets for augmented rFVIIa-induced haemostasis.

Table 3. Coagulation activation parameters obtained from patients treated by an individually tailored dose of combined concomitant therapy, before (time: 0 h) and 0.5, 2, 4 or 6 h posttherapy administration.

Normal range	Time (hour)	D-Dimer (<200 ng mL ⁻¹)	TAT (1–4.1 µg L ⁻¹)	F1 + 2 (69–229 pmol L ⁻¹)
Patient 1	0	136	2.55	203
	0.5	123	15.4	>1200
	2	146	7.36	1200
	4	144	5.93	832
Patient 2	0	<50	2.18	100
	0.5	<50	11.33	1183
	2	<50	4.26	620
	6	73	2.35	169
Patient 3	0	320	2.42	117
	0.5	195	10.19	1102
	2	290	4.13	605
	4	321	2.17	139

These reports demonstrated improved TG with higher concentrations of rFVIIa combined with either prothrombin, FIX or FX or phospholipids [17,22–24], all components of FEIBA. Thus, timely administration of all components (namely: CCT) might be beneficial at lower doses when compared to sequential therapy.

Though our study has some limitations, including the fact that TG was not systematically assayed and not compared with *ex vivo* results for either rFVIIa or FEIBA alone, we believe that our findings offer an alternative therapy option for 'tough to treat' patients with inhibitors.

Therapy cost is an important consideration using bypassing agents for inhibitor patients and the cost of treating individual patients may greatly magnify the cost of treating the inhibitor population in general [25]. The use of lower than standard drug doses and the single administration required, may lead to significant cost reduction. Our patients were treated by CCT as a first line treatment, saving the consequences of prolonged bleeding as well as the cost of repeated doses.

The main concern of haemophilia treaters who use bypassing agents is their fear of potential thrombotic complications. Thrombotic events, although rare, may occur with either of both products [26,27]. Thrombosis occurs mainly in patients with preexisting risk factors [28,29]. Kraut *et al.*, in their surgical cohort of high-titre inhibitor patients, reported one case of DIC in a patient with acquired haemophilia [20]. In a case-report of sequential administration of FEIBA and rFVIIa, one patient developed pulmonary embolism [30]. In our study, no thromboembolic complications or any serious adverse events were reported with CCT. No overt DIC was documented by us, though mild elevation of D-Dimers was noted in association with the minor surgical procedure performed in one of our patients. The transient increase of TAT and F1 + 2 noted, and previously reported [19], may simply represent the haemostatic effect of both therapies. Whereas no clinical thrombosis was manifested in our patients, since no imaging studies were routinely performed to exclude 'silent' thrombotic episodes, the potential risk of thromboembolism following use of CCT should not be ignored.

In conclusion, CCT with low doses of rFVIIa and APCC seems to be a promising mode of treatment for haemophilia patients with inhibitors, especially (but not limited to) patients that become refractory to each drug alone. The laboratory activation of coagulation markers, albeit not associated with clinical thrombosis in our patients, merits special caution

when such therapy is applied. Controlled trials with a larger number of patients are required to further assess the safety, efficacy and cost benefit of this treatment.

Disclosures

U. Martinowitz and G. Kenet have received honoraria for speaking in conferences, took part in advisory boards and served as PI in research projects supported by Novo Nordisk. The other authors stated that they had no interests which might be perceived as posing a conflict or bias.

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