Brief report

Prospective assessment of thrombin generation test for dose monitoring of bypassing therapy in hemophilia patients with inhibitors undergoing elective surgery

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Clinical response to bypassing agents (BPAs) may vary between patients. Surgery is a particular situation, requiring effective hemostasis during the procedure and for several days postoperatively to obtain satisfactory wound healing. However, the optimal dose of BPA in different surgical situations has not been clearly established. We report here a prospective assessment of thrombin generation test (TGT) in monitoring the effectiveness of BPA during 10 elective invasive procedures performed in 6 patients with severe hemophilia and high-titer inhibitors. A standardized 3-step protocol was used in all cases to individually tailor BPA. Thrombin-generating capacity of patients increased after in vitro and ex vivo addition of BPA in a dose-dependent manner. Our results also showed a correlation between in vivo clinical response to BPA and thrombin-generating capacity. These data suggest that TGT may represent a surrogate marker for monitoring bypassing therapies in surgical situations. (*Blood.* 2010;116(25):5734-5737)

Introduction

The development of inhibitors is one of the most serious complications of hemophilia, and bleeding episodes are more difficult to control in these patients.[1] Several studies have reported excellent efficacy of FEIBA (Baxter) and NovoSeven (NovoNordisk) in 80% to 85% of cases.[2-4]Nevertheless, the presence of an inhibitor demands extremely cautious surgical management of patients in a multidisciplinary environment with appropriate surgical technique and effective hemostatic control. Moreover, the clinical response to BPA may vary among patients. The optimal use of BPA is hampered by a lack of laboratory assays to monitor efficacy and determine adequate dosing. Recently, we described the first application of TGT in a surgical setting, showing that the assay might be useful in guiding the choice of the most effective therapeutic option in inhibitor patients.[5] We report now a prospective clinical assessment of TGT and our results show a correlation between thrombin generation (TG) capacity and the clinical outcome of patients.

Methods

Subjects and surgical procedures

The correlation between the clinical and biologic efficacy of BPA measured by TGT was prospectively evaluated in 10 surgical procedures. Six patients with severe hemophilia A and high-titer inhibitors (> 5 BU/mL) undergoing elective surgeries were treated and documented after giving informed consent in accordance with the Declaration of Helsinki. The study was approved by the Lyon University Hospital's ethical committee.

The control population comprised 96 healthy males (mean age ± SD, 40.2 ± 11 years) not using drugs known to affect the coagulation system and without history of bleeding or thrombosis.

Study design

Dose tailoring of BPA was performed using a standardized 3-step protocol including (1) in vitro spiking experiments evaluating the TG ability of increasing concentrations of NovoSeven (0, 90, 180, 200, 240, and 270 μg/kg) and FEIBA (0, 75, and 100 U/kg) to determine the minimum dose of each BPA that normalizes TG capacity; (2) an ex vivo confirmation step in which TG is measured before and after the administration of the BPA, which gave the best hemostatic profile in the previous in vitro spiking experiment using the dose which fully normalized in vitro TG; and (3) monitoring of the chosen dose of the BPA during the surgery and postoperative period.

As NovoSeven induces factor (F) Xa and FIXa generation on activated platelets,[6,7] the hemostatic efficacy of NovoSeven was evaluated using platelet-rich plasma (PRP).[8] FEIBA, having a different mechanism of action, was evaluated using platelet-poor plasma (PPP).[9]

Blood and PPP and PRP samples

Venous blood was collected into citrated Monovette tubes (Sarstedt) loaded with 1.45μM corn trypsin inhibitor (Haematologic Technologies). PPP and PRP were obtained as previously described.[10-12]

Routine measurements

FVIII activity was measured using BioMerieux-deficient FVIII kit (Marcy l'Etoile). Anti-FVIII antibody levels were determined by the Bethesda assay as previously described.[13]

TG measurement

TG was measured using the calibrated automated TGT (CAT; Thrombinoscope bv) and a Fluoroscan Ascent fluorometer (Thermolabsystems OY) as previously described,[10-12] using TF1pM and phospholipids 4μM (final concentrations). PRP samples were tested with TF1pM only. Results were available in 3 hours after blood drawing. The analysis of the main

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Table 1. Patient characteristics

Patient no.	Age, y	FVIII:C (IU/dL)	Inhibitor (BU/mL)	Clinical BPA response before TG testing
1	42	< 1	21	Bad responder to APCC; weak efficacy of rFVIIa
2	50	< 1	18	Good responder to APCC
3	36	< 1	9	Good responder to APCC
4	40	< 1	78	Bad responder to both APCC and rFVIIa
5	71	< 1	10.5	Bad responder to APCC; weak efficacy of rFVIIa
6	33	< 1	24	Good responder to rFVIIa; weak efficacy of APCC

Table 2. In vitro spiking experiments (step 1)

	ETP (nM.min)						
	rFVIIa				APCC		
Patient no.	0 μg/kg (PRP)	90 μg/kg (PRP)	200 μg/kg (PRP)	270 μg/kg (PRP)	0 U/kg (PPP)	75 U/kg (PPP)	100 U/kg (PPP)
1	315	1067	1315 (normalized)	1365 (normalized)	289	883	1012
2	410	1650 (normalized)	2337	3004	356	1378 (normalized)	2676
3	164	805	1004	1051	110	1226 (normalized)	1526 (normalized)
4	188	283	344	447	197	634	898
5	196	462	826	844	186	889	930
6	485	1530 (normalized)	1650 (normalized)	1943 (normalized)	399	1004	1054

TGT parameters, peak, endogenous thrombin potential (ETP), and TG rate, showed a certain correlation between clinical bleeding phenotype and both ETP and TG rate. However the large variability of TG rate in normal control PRPs makes it difficult to reliably determine normal values for this parameter. The peak cannot be chosen as the peak values obtained in PRP cannot be compared with those obtained in PPP. Therefore, in this study we used ETP as the main biologic parameter.

Results and discussion

Normal ETP values determined in 96 controls were 1487 ± 186nM.min (mean ± SD) in PPP and 1544 ± 178nM.min (mean ± SD) in PRP.

Tables 1 through 4 summarize patients' characteristics, surgeries, ETP obtained with the 3-step protocol, BPA regimen, and clinical outcome for each patient. TG was systematically measured before and one hour after each infusion of BPA during the surgery and every morning before a new infusion, to evaluate the residual TG activity of the patient. Intraoperative blood loss was quantified by measuring irrigation fluid and weighing surgical sponges used for blood and fluid collection during surgery.

Case 1

The patient was clinically a bad responder to FEIBA and he reported a weaker efficacy of NovoSeven 90 μg/kg during his last bleeding episode. According to TGT results, the surgery was performed using NovoSeven 200 μg/kg. Another high dose of 200 μg/kg was infused 2 hours later, followed by the usual regimen of 90 μg/kg every 2 hours. On the second postoperative day NovoSeven 90 μg/kg was given every 3 hours before starting continuous infusion with a dosage of 20 μg/kg/h. The patient exhibited a bleeding at postoperative day 3, after the modification of NovoSeven regimen. At this time, we observed a significant decrease in both ETP and hemoglobin, suggesting a correlation between TG capacity and clinical bleeding risk.

Table 3. Ex vivo confirmation (step 2)

Patient no.	Chosen BPA	Chosen dose	ETP 1 h after infusion (nM.min)
1	rFVIIa	200 μg/kg	1193 (normalized)
2	APCC	75 U/kg	1532 (normalized)
3	APCC	75 U/kg	1434 ± 212 (mean ± SD, n = 4) (normalized)
4	APCC	75 U/kg	763 (low ETP)
5	rFVIIa	120 μg/kg	892 (low ETP)
6	rFVIIa	90 μg/kg	1546 (normalized)

Case 2

In vitro spiking experiments showed a similar satisfactory efficacy of both NovoSeven and FEIBA. The patient's usual home treatment was FEIBA, for which the ex vivo assessment confirmed hemostatic efficacy. Surgery was performed under FEIBA 75 U/kg given every 8 hours, with no excessive bleeding.

Case 3

The patient underwent 4 orthopedic procedures during the study period. The standardized 3-step protocol was performed before each surgery. Results were convergent and they showed a better efficacy of FEIBA in this particular patient. Ex-vivo TG measurements performed before each surgery confirmed a complete correction of ETP with FEIBA 75 U/kg. The patient underwent the 4 surgeries with FEIBA 75 U/kg every 8 hours with satisfactory clinical efficacy.

Case 4

The patient was a poor responder to both FEIBA and NovoSeven, with unsatisfactory bleeding control with these drugs. In vitro and ex vivo TG measurements were performed before the surgery and confirmed the insufficient correction of TG in this particular patient with both FEIBA and NovoSeven. With knowledge of these results and considering the extremely high risk of the surgery, we designed

Table 4. Monitoring during surgery (step 3)

Patient no./surgical procedure	Treatment regimen	ETP during surgery (nM.min)	Post-operative ETP (nM.min)	Intra-operative blood loss (mL)	Hemoglobin (g/dL) before/after surgery	Clinical outcome
1						
Transfemoral amputation of the left lower limb	D0: rFVIIa 200 μg/kg	1567 (normalized)	D0: 1256	300	D0: 14/11.5	No excessive bleeding during surgery
	D1: rFVIIa 90 μg/kg/2H		D1: 1312			
	D2: rFVIIa 90 μg/kg/3H		D2: 865		D2: 6.5	Hematoma into left adductor magnus (ultrasound)
2						
Left total ankle arthroplasty	APCC 75 U/kg /8H	1440 (normalized)	D0: 1236; D1: 1416; D2: 1139	500	15/12	No excessive bleeding and satisfactory wound healing
3						
Right elbow synovectomy	APCC 75 U/kg /8H		D0: 1600 ± 128	170	13/12	No excessive bleeding and satisfactory wound healing
Left total knee arthroplasty	APCC 75 U/kg /8H	1417 ± 184 (mean ± SD; n = 4)	D1: 1238 ± 103	600	12/9.8	
Right ankle replacement	APCC 75 U/kg /8H	(normalized)	D2: 1205 ± 111	250	12.8/11.5	
Right total knee arthroplasty	APCC 75 U/kg /8H			450	13/11	
4						
Bilateral total knee arthroplasty	Immunoadsorption; FVIII concentrate; APCC 75 U/kg at D4	1027 (low ETP)	D0: 957; D2: 884; D4: 1009	600 × 2	12/8	No excessive bleeding and satisfactory wound healing
5						
Cataract surgery	rFVIIa 120 μg/kg	956 (low ETP)	D0: 938; D1: 654	0	13/13.1	No excessive bleeding and satisfactory wound healing
Partial colectomy	D0-D4: rFVIIa 120 μg/kg/2H	832	D0-D4: 752 ± 92	NA	D0: 10.5/8.2	Death by excessive bleeding
	D5: APCC 100 U/kg/8H	(low ETP)	D5: 869		D5: 6.3	
6						
Left elbow synovectomy	rFVIIa 90 μg/kg/2H	1903 (normalized)	D0: 1935; D1: 1789; D2: 1566	< 100	12.5/12	No excessive bleeding and satisfactory wound healing

a strategy that included immunoadsorption to eliminate the inhibitor, substitution with FVIII and administration of FEIBA when the anamnestic response occurred. The monitoring of this successful strategy using TGT throughout the perioperative period was previously described in a case report.[5]

Case 5

The patient underwent 2 surgical procedures during the study period. Before the cataract surgery, in vitro spiking experiments showed insufficient TG correction with FEIBA and NovoSeven at all tested concentrations. However, a partial correction was observed in the presence of NovoSeven 120 μg/kg (ETP = 815 nM.min), with a plateau reached beyond 120 μg/kg. He underwent the cataract surgery with NovoSeven 120 μg/kg. No bleeding occurred during the surgery, which is usually not related to a high risk of bleeding. Eight months later, he was admitted to the emergency department for acute rectal bleeding. He was first treated with NovoSeven 90 μg/kg every 2 hours; he experienced continuous bleeding despite NovoSeven infusions. Colonoscopy identified a dissecting intramural hematoma of the sigmoid colon. The patient underwent urgent bowel resection. The surgery was performed after an infusion of NovoSeven 120 μg/kg, which was repeated every 2 hours for 48 hours. The patient exhibited life-threatening bleeding despite bypassing therapy and he was intensively transfused. On the fifth postoperative day rebleeding occurred. CT scan demonstrated concomitant intraperitoneal and intrapleural bleeding. The patient's condition rapidly deteriorated and NovoSeven was stopped and replaced by FEIBA 100 U/kg. In parallel, the patient was intensively transfused, but clinical efficacy could not be obtained. The patient developed disseminated intravascular coagulation (DIC), and death occurred the day after as a result of continuous hemorrhage. In this particular patient neither NovoSeven nor FEIBA could achieve effective hemostasis as demonstrated by deficient TG. The concomitant use of both drugs was not tested in this patient because of DIC.[14]

Case 6

Before synovectomy, in vitro spiking experiments showed a better efficacy of NovoSeven 90 μg/kg compared with FEIBA 75 U/kg. Ex vivo results confirmed the hemostatic efficacy of NovoSeven 90 μg/kg, which normalized the ETP values one hour after infusion. The synovectomy was performed after an infusion of NovoSeven 90 μg/kg. No bleeding complication occurred.

The treatment and monitoring of BPA is still challenging, and there is a need for a reliable biomarker that correlates with the clinical outcome of patients. This study was designed to prospectively assess a 3-step protocol using TGT, which aims to individually tailor and monitor BPA in surgical situations. We observed that in patients with normalized ETP, no bleeding complication occurred. We conclude that TGT results correlate with the surgery-related clinical bleeding risk and ETP may be used as a surrogate marker for monitoring BPA. Careful standardization of the preanalytical and analytical test conditions is required before a wider application of the assay in clinical laboratories.[15]

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Authorship

Contribution: Y.D. designed, performed the research project and wrote the manuscript; A.L. performed the study; and C.N. designed the project and revised the manuscript.

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