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Combination of hemostatic therapies for treatment of patients with hemophilia A and inhibitors

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ABSTRACT

Background: Therapy application and monitoring of patients with hemophilia A (HA) and inhibitors are challenging. In the current study, combined FVIII – bypass therapy was implemented for a cohort of severe HA patients with inhibitors.

Methods: Plasma of 15 HA patients with inhibitors was spiked ex vivo with FVIII, rFVIIa, FEIBA and their combinations and thrombin generation (TG) was studied. Some patients who experienced hemarthroses or required minor surgeries were treated by a combined concomitant administration of FVIII + FEIBA as IV bolus doses.

Results: TG spiking studies showed individual responses not correlated to inhibitor titer. Combinations of agents augmented TG as compared to any single agent, while combined FVIII + FEIBA yielded the highest TG, supporting it as a potential treatment. Following emergent successful surgery of child treated by concomitant FVIII + FEIBA, a total of 396 episodes in 7/15 patients were treated with concomitant FVIII + FEIBA. Five patients were treated for bleeding episodes only, whereas 2 were children undergoing immune tolerance induction (ITI) with FEIBA prophylaxis. Four minor surgeries were performed on FVIII + FEIBA repeated infusions. Neither thrombosis nor any other adverse events were documented.

Conclusion: A combination of FVIII + FEIBA may be effective and safe as an alternative treatment option for some high-responding inhibitor patients.

1. Introduction

Hemophilia A (HA) is a congenital severe bleeding disorder that may be treated by replacement FVIII therapy [1,2]. About 30% of severe HA patients develop inhibitory antibodies against FVIII. Formation of antibodies directed against the missing factor concentrate is a complication that may render the standard anti- hemophilic care ineffective. Immune tolerance induction (ITI) may overcome this complication and eradicate the inhibitory antibodies in the majority of HA patients. However, regardless of ITI, bleeding episodes occur and should be treated. Bypass agents [recombinant activated factor VII (rFVIIa) or activated prothrombin complex concentrate (APCC) e.g. FEIBA] may be required to control hemostasis, especially for high-responding patients [HR = inhibitor titer above 5 Bethesda Units (BU)] [3,4]. There is high variability in these patients' bleeding tendency in addition to the fact that the inhibitor titer does not reliably predict their hemostatic responses Therefore, there is a need to individually tailor treatment for this unique group of patients.

Monitoring therapy in HR inhibitor patients treated by bypass agents is extremely challenging. Global assays such as thrombin generation (TG) and thromboelastometry have been used as complimentary tools that enable evaluation of clot formation, clot strength, fibrinolysis and support treatment decisions [5–7].

Various studies have demonstrated the use of TG as a tool guiding therapy in patients with severe HA and inhibitors [7–14]. It is our current practice to evaluate TG (with several ex vivo spiking treatment option combinations) for all our inhibitor patients. Combinations of rFVIIa and FEIBA or FVIII and rFVIIa have been reported to augment TG and improve hemostasis in difficult to treat inhibitor patients [3,9,15–18]. Surprisingly, FVIII + FEIBA treatment for bleeding episodes or surgeries in non ITI inhibitor patients has rarely been reported to date. Combined or sequential administration of FVIII together with FEIBA has been long reported during ITI [19]. Furthermore, repeated publications addressed FEIBA prophylaxis for HR inhibitor patients

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[8,20–24]. In vitro studies have demonstrated augmented TG while combined FEIBA and FVIII were spiked using inhibitor plasma [14,25,26].

The impetus for this study was a young boy with familial HA and inhibitor (peak measured at 186 BU). Unfortunately, developmental delay required cranial magnetic resonance imaging (MRI), disclosing a tectal tumor (most probably low grade glioma) with increased intra cranial pressure. While attempting to tailor his optimal peri-surgical therapy, TG studies revealed that combined FVIII + FEIBA may induce the best hemostasis as compared to other options. This prompted the design of a comprehensive study including the patient cohort presented below.

Herein we summarize our laboratory and clinical data regarding various combined potential treatments for our inhibitor patients. The novel option of combined FVIII + FEIBA therapy in non- ITI inhibitor patients will be presented and discussed for the first time.

2. Materials and methods

2.1. Patients and ethics

Fifteen severe HA patients (FVIII < 1%) with inhibitors, part of our patient population followed in our center, were studied. Inhibitor patients who were followed-up at least every 3 months in addition to having a well-documented bleeding log diary were eligible for study enrollment. Patients with concomitant coagulopathies (e.g., von Willebrand disease) and patients treated by antiplatelet agents were excluded. Demographic data and characteristics of our heterogeneous patient cohort are presented in Table 1. Laboratory studies of all patients, were performed during routine clinic visits. Control plasma samples for laboratory assays were obtained from 24 healthy male volunteers. All subjects or their guardians gave their informed consent to blood drawing for study purposes. The study was approved by the institutional ethical committee in compliance with the Declaration of Helsinki.

2.2. Reagents

rFVIIa was purchased from NovoNordisk, Bagsvaerd, Denmark. Recombinant FVIII (Kogenate® FS) was purchased from Bayer, Bayer Schering Pharma, Leverkusen, Germany. FEIBA was purchased from Baxter, Vienna. The reagents were prepared according to the manufacturer's instructions.

2.3. Processing of blood samples

Blood samples were drawn in 0.109 M buffered citrate tubes. Platelet-poor plasma (PPP) was obtained at room temperature by centrifugation of blood at 2000*g* for 10 min. The plasma was then aspirated into a plastic tube, and residual platelets were removed following a further centrifugation at 14000*g* for 5 min. The double-spun PPP was stored in aliquots at −70 °C and thawed prior to assay.

2.4. Thrombin generation

TG was measured in PPP as described previously [10,27]. Twenty microliters of working buffer containing 4 μM phospholipid (MP-Reagent, Stago, Gennevilliers, France) were placed in round-bottom 96-well plates. Eighty microliters of plasma were added to the buffer. FEIBA, rFVIIa and FVIII alone or in combination were spiked into the plasma. TG was initiated by adding 20 μL of fluorogenic substrate/$CaCl_2$ buffer (FluCa-kit, Stago). Fluorescence was measured with a fluorometer (Fluoroskan Ascent, Lab system, Helsinki, Finland) with an excitation filter at 390 nm and an emission filter at 460 nm. The results were displayed as plots and derived parameters, i.e., lag time (min), endogenous thrombin potential (ETP, nM·min) and peak height (nM), calculated by dedicated computer software attached to the fluorometer (version 3.0.0.29 Thrombinoscope-BV). All experiments were repeated by the same researcher.

2.5. Statistical analysis

Data was expressed as median and interquartile range and analyzed using the Kruskal–Wallis test followed by Dunn's post hoc test to compare patient samples with control samples. The difference was considered to be statistically significant if the p value was < 0.05.

3. Results

The first patient was a 20 month old boy with familial severe HA due to inversion 22 mutation, in whom FVIII inhibitor evolved after eight exposure days to recombinant FVIII on demand therapy. As MRI disclosed increased intra cranial pressure, attributed to a tectal mass, an urgent ventriculo-peritoneal (VP) shunt was required. Considering the presence of brain tumor (most probably low grade glioma of the tectum) with increased potential for intra cranial hemorrhage (ICH) in a patient with HR inhibitor, we aimed at maximal hemostasis around this surgical intervention. TG studies were performed on patient's PPP with ex vivo spiking of FVIII and bypass agents. Fig. 1A clearly shows the advantage of FVIII + FEIBA combination over other potential

Table 1
Demographic data and characteristics of the patients.

Patient number #	Age (years)	Range of BU (BU in analysis)	Mutation	Target joint	Bleeding frequency
1	1	< 0.5–21 (4)	Deletion exon 8 + 9	No	2–3/month
2	3.5	0–180 (19)	Frame shift exon 14	No	1/week
3	4	0.5–284 (17)	Inv 22	No	1–2/week
4	4.5	1.5–92 (8)	None per exon seq	No	1–2/month
5	38	< 0.5–9 (9)	No inv	Ankle, elbow, knees	1/month
6	72	8–80 (58)	Inv 22	Knee, elbow	1/month
7	3	< 0.5– > 30 (8)	None per exon seq	No	1–2/month
8	40	9–56 (14)	None per exon seq	Elbow, knees	2–3/ month
9	6	1.5–104 (12)	NA	No	1/month
10	6.5	0.4–668 (9)	Inv 22	No	1–2/month
11	6	24–800 (62)	None per exon seq	Knee, ankle	2–3/week
12	50	8–80 (34)	No inv	Knee elbow	1–2/month
13	30	104–800 (620)	Del exon 13–21	Ankle, elbow	1–2/week
14	1.5	1–420 (64)	Inv 22	No	2–3/month
15	25	4–21 (11)	None per exon seq	Elbow	2–3/month

BU - Bethesda Units; inv - inversion; seq - sequence.