Thrombosis Research 178 (2019) 112–118

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

In vitro assessment of edoxaban anticoagulant effect in pediatric plasma

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ARTICLE INFO

Keywords:
Coagulation
Edoxaban
Pediatric
Thrombin generation

ABSTRACT

Introduction: Anticoagulant therapy in pediatric patients remains an issue and safer therapies, such as direct oral anticoagulants could overcome the limitations of conventional anticoagulant treatments in this population. Edoxaban, a factor Xa inhibitor, is used for the prevention and treatment of venous thromboembolism. Due to its pharmacokinetic characteristics, edoxaban is a promising candidate molecule for children. This study compared edoxaban *in vitro* effect in children and adults.

Materials and methods: Blood samples were prospectively collected from 87 adults and 97 children (n = 12: < 2 year-old; n = 8: 2–4 year-old; n = 9: 5–7 year-old; n = 14: 8–9 year-old; n = 10: 10–13 year-old; n = 15: 14–15 year-old; and n = 29: 16–18 year-old). Plasma samples were supplemented *in vitro* with edoxaban to a final concentration of 50, 150 or 300 ng/mL, and then edoxaban effect on prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen (Clauss assay), specific anti-factor Xa activity and thrombin generation assay (TGA) (with 5pM tissue factor and 4 nM phospholipids) was evaluated.

Results: PT, aPTT, and specific anti-Xa activity exhibited similar dose-dependent responses to edoxaban in the different age groups. The reduction of thrombin peak, the most edoxaban-sensitive TGA parameter, was similar in adults and children, but for the youngest group (< 2 year-old) where the peak value reduction (median [Q1–Q3]) was higher than in adults (51% [44–59] *versus* 40% [32–46], $p < 0.01$; 74% [63–80] *versus* 65% [58–70], $p < 0.05$; and 84% [73–88] *versus* 76% [70–80], $p < 0.05$ for 50, 150 and 300 ng/mL edoxaban, respectively).

Conclusions: Edoxaban *in vitro effect* are comparable in children and adults except in the < 2-year-old group.

1. Introduction

The incidence of venous thromboembolism (VTE) in hospitalized children has strongly increased during the last decades, regardless of their age class [1,2]. The most common anticoagulant therapies used in this population are multi-target compounds, such as unfractionated heparin (UFH), low molecular weight heparins (LMWH) and vitamin K antagonists (VKA) [3–5]. However, their use is associated with important drawbacks, particularly in pediatric populations. VKA management requires strict monitoring [6] and the availability of a liquid formulation. UFH treatment requires injections and monitoring because of the variable pharmacokinetic profile. Similarly, LMWH are not available in oral formulation and require a regular follow-up in pediatric populations. Their effect cannot be easily reversed with protamine [7,8].

The concept of developmental hemostasis should be integrated when an anticoagulant is required in pediatric patients. Several studies have shown that vitamin K-dependent factors (factor II, VII, IX and X) and contact-phase factors tend to gradually increase from birth and reach adult values around 6 months of age. Conversely, fibrinogen and factor V, VIII and XIII levels at birth are comparable with the adult levels. At birth, the levels of protein C, protein S and antithrombin are lower, whereas those of alpha-2-macroglobulin are higher compared with adults [9–16]. These proteins reach adult levels after few months or few years. Despite these differences in the concentration of coagulation factors, there is no evidence that children are more susceptible to

Abbreviations: PT, prothrombin time; APTT, activated partial thromboplastin time; ETP, endogenous thrombin potential; LMWH, low molecular weight heparin; TGA, thrombin generation assay; UFH, unfractionated heparin; VKA, vitamin K antagonists; VTE, venous thromboembolism

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https://doi.org/10.1016/j.thromres.2019.04.014
Received 10 December 2018; Received in revised form 5 March 2019; Accepted 12 April 2019
Available online 13 April 2019
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coagulation abnormalities, such as bleeding or thrombosis [17]. However, the effects of anticoagulant drugs on hemostasis are affected by these developmental differences [18], and this could lead to variable and potentially dangerous responses in pediatric populations. Importantly, these pharmacodynamic changes could be overlooked because monitoring is done using tests based on adult data that do not take into account these developmental differences.

Edoxaban tosylate (edoxaban) is a direct oral anticoagulant that inhibits factor Xa. In adults, edoxaban is indicated for reducing the risk of stroke and systemic embolism in patients with non-valvular atrial fibrillation [19] and for the prevention or treatment of deep vein thrombosis and pulmonary embolism after parenteral anticoagulants for 5–10 days [20]. Edoxaban is currently not approved for anticoagulation in patients younger than 18 years of age. However, its oral administration route, interaction with few drugs and food, and antithrombin level-independent anti-Xa inhibition are interesting features for pediatric use.

Here, we compared the hemostatic response and coagulation assay results in plasma samples from adults and children (different age classes) spiked *in vitro* with specific concentrations of edoxaban.

2. Materials and methods

2.1. Pediatric and adult populations

For this study, 87 adults (> 18 years of age) and 97 children (≤18 years of age) were prospectively enrolled at Clermont-Ferrand University Hospital between January 2016 and November 2017. This study was approved by the local ethics committee (AU765, Sud-Est VI France). Exclusion criteria for adults and children were: ongoing antiplatelet or anticoagulant therapy, personal history of bleeding or VTE, and coagulation disorders [fibrinogen < 2 g/L, prothrombin time (PT) > 17 s, activated partial thromboplastin time (aPTT) > 50 s]. Children were separated in different age groups to take into account the developmental coagulation changes: < 2 years, 2–4 years, 5–7 years, 8–9 years, 10–13 years, 14–15 years and 16–18 years of age.

2.2. Blood sampling and plasma preparation

Blood was collected by venipuncture in 0.109 M citrate tubes (Beckton Dickinson, le Pont de Claix, France) after discarding the first few milliliters of blood. Corn trypsin inhibitor, an inhibitor of contact phase was not added because coagulation in the thrombin generation assay (TGA) was initiated by addition of 5pM tissue factor, a concentration that makes irrelevant the inhibition of contact phase [21]. Platelet-poor plasma (PPP) required for TGA was obtained by centrifuging the blood samples twice (2500*g* at 20 °C for 15 min/each) with an intermediate decantation, according to the guidelines of the International Society on Thrombosis and Haemostasis (ISTH) [22]. Samples were stored at −80 °C until testing. Then, frozen plasma samples were thawed in a water bath at 37 °C for 5 min.

Edoxaban stock solution (1.0 mg/mL in dimethyl sulfoxide) was diluted with phosphate buffered saline (PBS) to 5000 ng/ml, 15,000 ng/ml and 30,000 ng/mL working solutions that were added to the plasma samples to reach the target concentrations (50, 150 and 300 ng/mL) with a constant 1/100 dilution. An equivalent volume of PBS was added to baseline samples (0 ng/ml).

2.3. Coagulation assays

Coagulation assays were performed with a STA-R Max coagulometer (Stago, Asnières-sur-Seine, France) using the following reagents (all from Stago): Neoplastin CI + ® for PT, PTT-A® for aPTT, and STA-Fibrinogen® for fibrinogen (Clauss method). Edoxaban levels in plasma were measured using an anti-factor Xa activity assay (STA®-Liquid Anti-Xa, Stago) and the STA®-Edoxaban Calibrator and STA®-Edoxaban Control on a STA®-R analyzer (Stago).

TGAs were performed using the CAT method [23] with a fluorometer (Fluoroscan Ascent, ThermoLab Systems, Franklin, USA) equipped with a dispenser. Coagulation was initiated with 5 pM tissue factor in the presence of 4 μM procoagulant phospholipids (PPP reagent®, Stago). For each sample, calibration was performed with the Thrombin calibrator® (Stago). All plates (Immulon 2HB, Waltham, USA) were incubated at 37 °C for 10 min before adding the fluorogenic substrate and $CaCl_2$ (FluCa-kit®, Thrombinoscope BV). All tests were performed in duplicate with a < 10% difference between endogenous thrombin potential (ETP) results. Raw data were analyzed using Thrombinoscope™. For each assay, ETP (nM·min) and thrombin peak (nM) were the primary endpoints. The first represents the whole thrombin generated in a plasma sample, and the second the highest thrombin concentration that can be generated and that is particularly affected by edoxaban. Edoxaban effect on ETP and thrombin peak was also expressed as percent inhibition that corresponded for each patient to (1 − (ETP in the presence of edoxaban divided by the patient's baseline ETP)) × 100.

2.4. Statistical analysis

Sample size was estimated according to (i) the CONSORT 2010 statement, extension to randomized pilot and feasibility trials and (ii) Cohen's recommendations, which define effect-size bounds as follows: small (ES: 0.2), medium (ES: 0.5) and large (ES: 0.8, “grossly perceptible and therefore large”). More precisely, an effect size > 1.2 (according to pilot data) could be highlighted for a two-sided type I error at 0.001 (correction due to multiple comparisons) and a statistical power > 80%, with at least 75 adults and 15 children by group. Effect-size of the difference between children and adults are expressed by coefficient of Hedges *g* [95% confidence interval].

Statistical analysis was performed using the Prism software, version 6 (GraphPad software, Inc., La Jolla, USA). Tests were two-sided, with a type I error set at $\alpha = 0.05$. Continuous data were presented as medians [Q1–Q3]. Independent groups were compared using ANOVA, or the Kruskal-Wallis test when the ANOVA conditions were not met (normality and homoscedasticity verified with the Bartlett test). Dependent groups were compared using ANOVA or the Friedman test, followed by the appropriate multiple-comparison post-hoc, Tukey-Kramer, or Dunn test.

3. Results

Eighty-seven adults and 97 children (< 2 years of age: $n = 12$; 2–4 years: $n = 8$; 5–7 years: $n = 9$; 8–9 years: $n = 14$; 10–13 years: $n = 10$; 14–15 years: $n = 15$; and 16–18 years: $n = 29$) were included in the study. Their demographic characteristics are summarized in Table 1.

3.1. Routine coagulation and specific anti-Xa assays

Overall (pediatric and adult samples together), the impact of edoxaban on clotting times increased in a dose-dependent manner. PT ($n = 56$) increased from 13.3 s [12.7–13.9] to 14.2 s [13.5–15.3], 17.0 s [16.4–18.5] and 21.7 s [20.1–23.0] for vehicle, 50, 150 and 300 ng/mL edoxaban, respectively, and aPTT ($n = 59$) from 35.5 s [32.6–39.8] to 38.8 s [34.7–43.4], 44.2 s [39.5–50.3] and 50.3 s [43.7–56.1], respectively. Although the PT and aPTT values showed a linear response to edoxaban, they were relatively insensitive to the increasing concentrations of edoxaban, as indicated by their 1.6-fold and 1.4-fold increase, respectively, from baseline to 300 ng/mL edoxaban. Conversely, edoxaban had no effect on fibrin clot formation (fibrinogen assay) ($n = 50$) with fibrinogen concentrations of 3.2 g/L [2.7–4.0], 3.3 g/L [3.0–3.9], 3.3 g/L [2.9–3.9], and 3.2 g/L [2.7–3.9] for vehicle, 50, 150 and 300 ng/mL edoxaban, respectively.

Table 1
Summary of the participants' demographic characteristics.

	< 2 y	2–4 y	5–7 y	8–9 y	10–13 y	14–15 y	16–18 y	Adults
n =	12	8	9	14	10	15	29	87
Age	Months				Years			
Median	14	3	7	9	11	15	17	56
Range	3–23	2–4	5–7	8–9	10–13	14–15	16–18	21–87
Sex, n (%)								
Female	3 (25)	2 (25)	1 (11)	7 (50)	3 (30)	11 (73)	17 (59)	41 (47)
Male	9 (75)	6 (75)	8 (89)	7 (50)	7 (70)	4 (27)	12 (41)	46 (53)

Y: years.

Table 2
Results of coagulation assays using plasma samples from adults and children before and after addition of edoxaban.

	< 2 y	2–4 y	5–7 y	8–9 y	10–13 y	14–15 y	16–18 y	Adults	*p*
PT (s) n =	3	4	5	4	6	8	9	17	
Vehicle	13.5 [12.7–13.6]	13.5 [12.3–14.7]	12.9 [12.6–15.3]	12.5 [12.4–12.7]	13.7 [13.0–14.6]	13.9 [13.5–14.1]	13.6 [13.2–14.9]	13 [12.7–13.5]	0.10
Edoxaban 50 ng/mL	14.3 [12.5–15.2]	15.0 [12.8–15.7]	13.6 [12.7–16.6]	13.2 [12.7–13.9]	15.1 [13.8–16.0]	14.8 [14.0–15.4]	14.7 [14.1–16.5]	14.1 [13.3–14.7]	0.34
Edoxaban 150 ng/mL	16.8 [15.2–17.9]	17.6 [15.4–20.4]	16.2 [14.9–20.9]	15.7 [14.7–16.4]	18.3 [16.3–20.1]	18.2 [17.0–18.9]	18.0 [16.7–21.2]	16.9 [16.6–17.8]	0.36
Edoxaban 300 ng/mL	22.7 [20.0–23.0]	21.2 [18.0–23.9]	21.0 [18.8–28]	20.0 [19.1–22]	23.3 [20.5–26.4]	22.6 [21.4–23.1]	22.3 [21.9–26.6]	21.2 [18.6–22.2]	0.60
APTT (s) n =	5	3	3	3	6	9	12	18	
Vehicle	40.4 [35.6–40.8]	35.1 [32.0–38.8]	37.7 [29.2–46.4]	35.5 [26.8–40.7]	37.7 [32.4–44.2]	35.6 [32.9–39.9]	36.4 [33.2–39.8]	35.1 [32.5–38.7]	0.87
Edoxaban 50 ng/mL	42.4 [36.3–44.1]	34.6 [34.3–43.2]	40.2 [29.6–50.5]	33.9 [27.2–47.2]	41.4 [36.0–47.7]	39.3 [35.6–44.7]	40 [35.9–43.7]	38.5 [35.0–42.4]	0.88
Edoxaban 150 ng/mL	48.4 [40.6–51.7]	41.2 [40.2–52.8]	41.6 [32.1–56.4]	34.8 [29.8–54.0]	46.4 [38.7–53.0]	45.9 [40.3–52.5]	45.6 [41.0–51.3]	44.6 [39.6–49.5]	0.96
Edoxaban 300 ng/mL	52.4 [47.3–56.9]	48.6 [42.9–55.8]	49.2 [38.2–62.9]	47.5 [32.5–65.1]	54.4 [42.8–61.4]	53.7 [43.6–63.5]	51.9 [46.3–58]	50.0 [43.8–54.7]	0.97

Data are the median [Q1–Q3].
PT: prothrombin time, aPTT: activated partial thrombin time, Y: years.
Edoxaban had no effect on fibrinogen level, and fibrinogen concentration was comparable in adults and children.

When these results were compared in the different age groups, PT, aPTT and fibrinogen concentration were comparable in the adult and pediatric populations before and after edoxaban addition and for all the drug concentrations (Table 2 and Fig. 1; available online).

When edoxaban level in plasma samples was measured using a specific anti-factor Xa assay, the concentrations were comparable to those expected on the basis of the *in vitro* spiking, without any age-related difference (Fig. 2; available online).

3.2. Thrombin generation assay

Overall (pediatric and adult samples together), edoxaban led to major changes in thrombin generation profile in a dose-dependent manner. The lag time, which corresponds to the time required to generate the first thrombin traces, gradually increased with edoxaban concentration. Lag time was sensitive to edoxaban anticoagulant effect, as indicated by the 3-fold increase for 300 ng/mL edoxaban compared with baseline. Similarly, time to peak significantly increased in a dose-dependent manner. ETP (maximum decrease of 40% at 300 ng/mL) and particularly thrombin peak (reduction by 80% at 300 ng/m) also were affected by edoxaban in a dose-dependent manner. The thrombin generation curve was altered by *in vitro* addition of edoxaban with a delay in the initiation phase, and a flattening of the curve leading to a plateau, especially from 150 ng/mL of edoxaban, due to the significant decrease in thrombin peak (Fig. 3). ETP was significantly and linearly affected by edoxaban concentration, whereas the thrombin peak decreased sharply at 50 ng/mL (approximately by 50%) and then less markedly (Fig. 4).

In the different age groups, at baseline, lag time ranged from 3.0 min [2.7–3.7] in the < 2-year age group to 3.2 min [2.7–3.7] in adults ($p = 0.128$). Similarly, baseline time to peak (about 6 min) hardly changed in the different age groups ($p = 0.31$). Conversely, ETP and thrombin peak were significantly influenced by age with lower values during the first years of life. Starting from 8 years of age, thrombin generation parameters were comparable in adults and children (Table 3).

In vitro addition of edoxaban, irrespectively of the concentration, did not differentially affect lag time and time to peak in the different age groups. Overall, for all age groups, including adults, lag time increased by 1.7-fold, 2.3-fold and 3.0-fold, and time to peak by 1.7-fold, 2.6-fold and 3.1-fold, upon addition of 50, 150 and 300 ng/mL edoxaban, respectively.

Lower ETP values during the first years of life were observed also after *in vitro* addition of edoxaban. Upon addition of 50 ng/mL edoxaban, ETP was 1296 nM·min [1144–1466] in adults, 979 nM·min [760–1099] in the < 2-year ($p < 0.001$, $g = -1.7[-2.5; -0.8]$ compared with adults), 976 nM·min [878–1239] in the 2 to 4-year ($p < 0.05$, $g = -1.3[-2.1; -0.3]$), and 964 nM·min [873–1083] in the 5 to 7-year age group ($p < 0.01$, $g = -1.5[-2.3; -0.6]$). After addition of 150 ng/mL edoxaban, ETP was 1108 nM·min [933–1225] in adults, 750 nM·min [496–946] in the < 2-year ($p < 0.001$, $g = -1.6[-2.4; -0.8]$), 815 nM·min [585–1027] in the 2 to 4-year ($p < 0.05$, $g = -1.2[-2.1; -0.3]$), and 861 nM·min [761–949] in the 5 to 7-year age group ($p < 0.05$, $g = -1.1[-1.8;-0.2]$). Finally, upon addition of 300 ng/mL edoxaban, ETP values ranged from 928 nM·min [692–1079] in adults to 572 nM·min [333–735] in the < 2-year ($p < 0.001$, $g = -1.5[-2.2; -0.7]$), 660 nM·min [490–823] in the 2 to 4-year ($p < 0.05$, $g = -1.1[-2.0; -0.2]$), and 702 nM·min

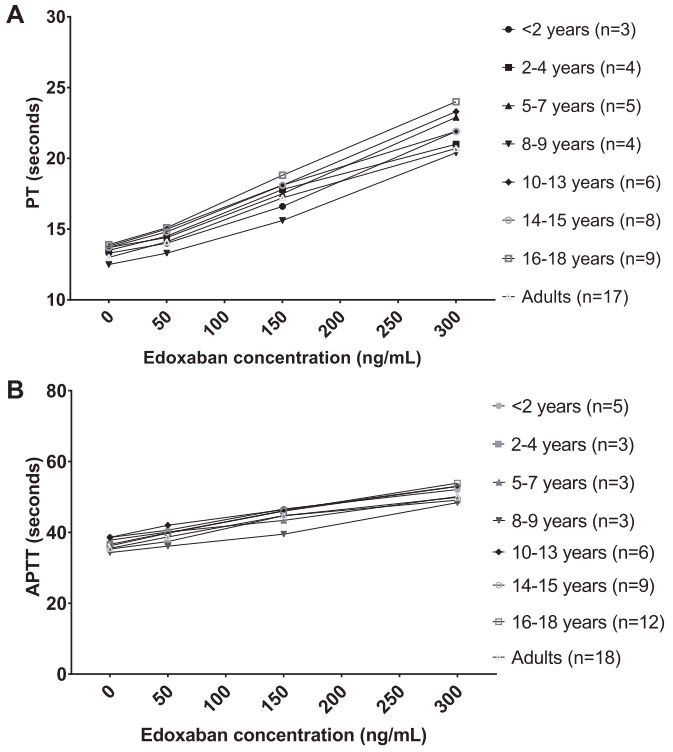

Fig. 1. Relationship between edoxaban concentration and coagulation assay results in the different age groups.
Edoxaban increased prothrombin time (PT) (A) and activated partial thromboplastin time (aPTT) (B) in a concentration-dependent manner. The relationship was linear and the sensitivity to edoxaban was age-independent. Edoxaban had no effect on fibrinogen level, and fibrinogen concentration was comparable in adults and children.

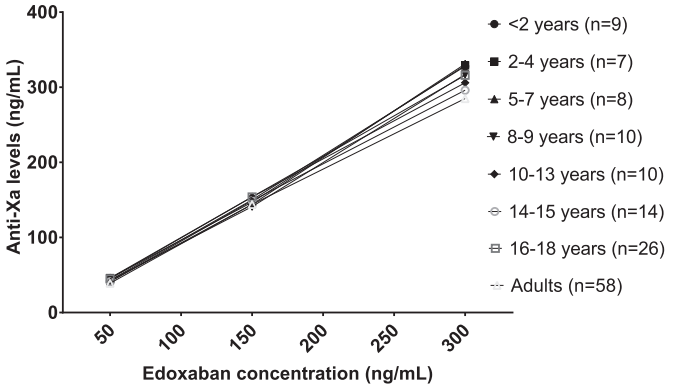

Fig. 2. Relationship between edoxaban concentrations and specific anti-factor Xa assay results in the different age groups.
Edoxaban level in measured with a specific anti-factor Xa assay in plasma samples spiked with at 50, 150 and 300 ng/mL. No influence of age was observed.

[611–814] in the 5 to 7-year age group ($p = 0.23$, g = −0.8[−1.6; −0.1]). Thrombin peak also was significantly lower in the < 2-year age group after *in vitro* addition of edoxaban (Table 3 and Fig. 4).

Edoxaban-mediated thrombin-generation inhibition was relatively stable in the different age groups. The percentage of ETP inhibition varied between 8 and 13% according to the age group ($p = 0.73$) for 50 ng/mL, between 25 and 35% ($p = 0.29$) for 150 ng/mL, and between 37 and 54% ($p = 0.08$) for 300 ng/mL edoxaban. The percentage of thrombin peak inhibition by edoxaban was similar between adults and children, but for the < 2-year age group where edoxaban-mediated

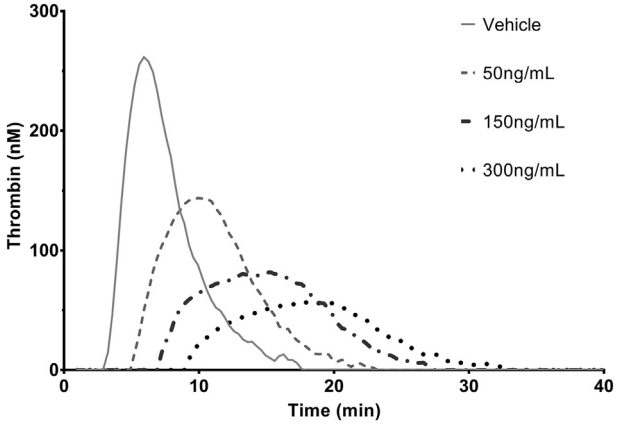

Fig. 3. Effect of increasing concentrations of edoxaban on thrombin generation. Thrombin generation assays were performed using platelet-poor plasma samples (with or without edoxaban) after addition of 5 pM tissue factor and 4 μM procoagulant phospholipids. Thrombin peak was the most sensitive parameter to edoxaban.

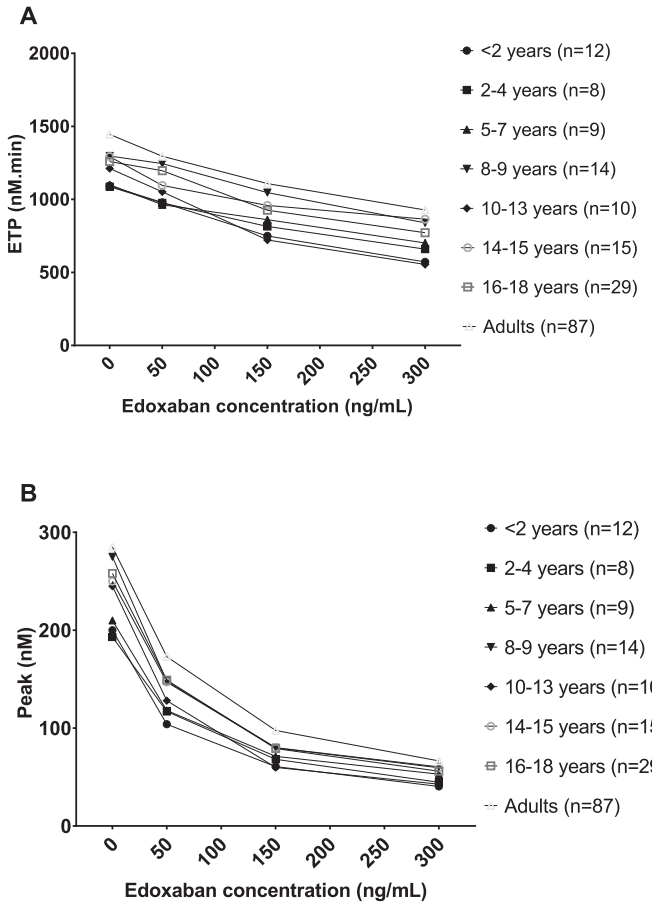

Fig. 4. Relationship between edoxaban concentrations and thrombin generation in the different age groups.
Edoxaban inhibited endogenous thrombin potential (ETP) (A) and thrombin peak (B) in a concentration-dependent manner.

inhibition was more pronounced than in adults (51% [44–59] *vs* 40% [32–46], $p < 0.01$, g = 1.0[0.3; 1.7]; 74% [63–80] *vs* 65% [58–70], $p < 0.05$, g = 0.4[0.2; 1.1]; and 84% [73–88] *vs* 76% [70–80], $p < 0.05$, g = 0.8[0.1; 1.4], for 50, 150 and 300 ng/mL edoxaban respectively) (Fig. 5). These results indicate that edoxaban affects ETP evenly across the different age groups, but has a stronger inhibition

Table 3
Thrombin generation assay results before and after edoxaban addition.

		< 2 yrs (n = 12)	2–4 yrs (n = 8)	5–7 yrs (n = 9)	8–9 yrs (n = 14)	10–13 yrs (n = 10)	14–15 yrs (n = 15)	16–18 yrs (n = 29)	Adults (*n* = 87)
LT (min)	Vehicle	3.0 [2.7–3.7]	3.1 [2.8–3.4]	3.0 [2.7–3.0]	3.0 [2.9–3.3]	3.2 [2.8–3.5]	3.0 [2.7–3.3]	2.7 [2.7–3.0]	3.2 [2.7–3.7]
	Edoxaban 50 ng/mL	5.1 [4.5–6.2]	5.6 [4.3–5.9]	5.2 [4.4–5.7]	5.0 [4.7–5.5]	5.7 [5.4–6.4]	5.3 [4.7–6.2]	5.2 [4.4–5.5]	5.2 [4.5–5.7]
	Edoxaban 150 ng/mL	7.7 [6.4–8.9]	7.6 [5.9–8.7]	7.0 [6.3–8.0]	7.0 [6.3–8.3]	8.8 [7.9–9.0]	7.6 [6.8–8.7]	7.3 [6.0–8.1]	7.0 [6.3–8.5]
	Edoxaban 300 ng/mL	9.6 [8.5–11.0]	9.3 [7.8–10.6]	8.7 [8.0–9.8]	8.7 [7.8–10.4]	10.9 [10.0–11.9]	9.7 [8.6–10.7]	9.2 [7.5–10.5]	9.0 [8.0–10.7]
ETP (nM·min)	Vehicle	1094 [897–1260]***	1087 [1012 – 1330]**	1101 [1038–1201]***	1297 [1237–1496]	1212 [1054–1459]	1293 [1080–1374]	1259 [1156–1695]	1445 [1276–1633]
	Edoxaban 50 ng/mL	979 [760–1099]***	976 [878–1239]*	964 [873–1083]**	1245 [1075–1344]	1052 [885–1369]	1096 [962–1302]	1197 [984–1505]	1296 [1144–1466]
	Edoxaban 150 ng/mL	750 [496–946]***	815 [585–1027]*	861 [761–949]*	1047 [870–1143]	722 [645–1163]	959 [729–1019]	926 [827–1198]	1108 [933–1225]
	Edoxaban 300 ng/mL	572 [333–735]***	660 [490–823]*	702 [611–814]	840 [684–967]	555 [423–944]*	865 [513–907]	772 [618–986]	928 [692–1079]
Peak (nM)	Vehicle	200 [170–262]***	193 [160–273]*	210 [190–243]*	275 [215–316]	245 [206–282]	250 [196–283]	258 [217–334]	285 [245–319]
	Edoxaban 50 ng/mL	104 [68–147]***	117 [96.6–198]	118 [90.5–139]*	148 [108–194]	128 [105–173]	147 [105–164]	149 [114–205]	173 [134–208]
	Edoxaban 150 ng/mL	61 [34–83]**	68 [49–91]	71 [54–90]	80 [66–110]	60 [55–102]	80 [60–95]	79 [59–110]	98 [75–127]
	Edoxaban 300 ng/mL	40 [20–56]**	45 [40–64]	53 [39–57]	60 [45–73]	43 [33–71]	60 [38–65]	56 [40–74]	67 [51–94]
TTP (min)	Vehicle	6.3 [5.4–7.3]	6.1 [5.4–8.5]	6.4 [4.7–6.7]	5.8 [5.4–6.7]	6.5 [5.3–7.5]	6.1 [5.1–7.3]	5.7 [5.1–6.2]	5.8 [5.2–6.5]
	Edoxaban 50 ng/mL	11.3 [9.46–13.3]	10.9 [8.77–11.7]	10.8 [8.17–11.4]	10 [8.92–11.1]	11.4 [10.2–12.2]	10.3 [8.9–12.2]	9.67 [8.7–11.9]	9.67 [8.5–11]
	Edoxaban 150 ng/mL	16.3 [13.2–17.8]	15.8 [13.3–16.3]	16.2 [11.2–16.9]	15.0 [13.8–15.6]	16.7 [15.6–18.1]	15.6 [14.3–18]	15.2 [12.5–16.8]	14.3 [12.3–16.3]
	Edoxaban 300 ng/mL	19.3 [16.3–22.3]	18.6 [16.3–19.3]	18.7 [14.8–20.2]	18.5 [16.2–19.3]	20.2 [19.5–21.2]*	18.8 [17.0–20.9]	18.5 [15.3–20.8]	17.5 [15.7–19.3]

Data are the median [Q1–Q3].
LT: Lag time; ETP: endogenous thrombin potential; TTP: Time to peak; yrs: years.
*** $p < 0.001$.
** $p < 0.01$.
* $p < 0.05$ compared with adults.

effect on the thrombin peak in young children.

4. Discussion

The present study investigated for the first time the *in vitro* effect of edoxaban, a direct and reversible factor Xa inhibitor, in different pediatric age groups and in adults. PT, aPTT, and the specific anti-Xa anticoagulant activity showed a similar dose-dependent response to edoxaban in the different age groups. Inhibition of thrombin peak, the most edoxaban-sensitive TGA parameter, was similar between adults and children, except for the youngest age group (< 2 years).

The recommended edoxaban dose in adults for prevention or treatment of deep vein thrombosis and pulmonary embolism (*i.e.*, 60 mg/daily) should give a maximum plasma concentration between 152 and 302 ng/mL, and trough plasma concentrations that rarely exceed 50 ng/mL [24]. Therefore, the plasma concentrations used in our study (*i.e.*, 50, 150 and 300 ng/mL) were adequate to the expected exposure in patients treated with the usual dose. Our results showed similar coagulation times in the different age groups for the classical coagulation tests (PT, aPTT and fibrinogen assay). These data are consistent with previous findings [12,15,16], although some studies found a variable lengthening according to age [9,13,14]. When we used conventional coagulation tests, edoxaban spiking led to PT increase in both adult and pediatric plasma samples in a concentration-dependent and linear manner, with low sensitivity (particularly at low concentrations). This effect could vary depending on the thromboplastin used. Similar results were obtained for aPTT. These data are comparable with edoxaban effects in adults [25–27]. Therefore, conventional assays, such as PT or aPTT, do not seem to be adapted to monitor the hemostasis changes during development. On the other hand, TGA is considered to be an innovative way to understand coagulation globally, especially when it is disturbed by complex changes that alter the coagulation balance, for instance in developmental hemostasis. Several studies indicate that TGA is a relevant tool to identify prothrombotic or hypocoagulable conditions [28–31]. TGA allowed highlighting the baseline decrease in thrombin generation in pediatric plasma samples, particularly the lower ETP and thrombin peak in children younger than 8 years of age. These data are consistent with several studies that reported a marked reduction in thrombin generation in children, including a correlation between age and ETP or thrombin peak [17,32–34].

Edoxaban inhibited thrombin generation in a dose-dependent manner. The thrombin peak appeared to be the most sensitive parameter with 50% inhibition with 50 ng/mL edoxaban, followed by a plateau at 150 ng/mL, while ETP inhibition was more linear. These findings are consistent with literature data on TGA in adults [35,36]. ETP was inhibited by edoxaban uniformly in all age groups. Conversely thrombin peak was the most age-sensitive parameter. Indeed, whatever the concentration, edoxaban anticoagulant effect was significantly higher in the < 2-year age group compared with adults. Therefore, in addition to basically generating less thrombin, these patients would be more sensitive to factor Xa inhibition than adults. Kremers et al. [33] showed in children a reduction in prothrombin conversion and a concomitant decrease in conversion rates with lower generation of prothrombinase complexes than in adults. Edoxaban may have a synergistic effect because it inhibits both free factor Xa and within the prothrombinase complex. These data should be taken into account for the definition of edoxaban doses in children.

One of the limitations of this study is the impact of alpha2-macroglobulin, the concentration of which is higher in neonates. Ignjatovic

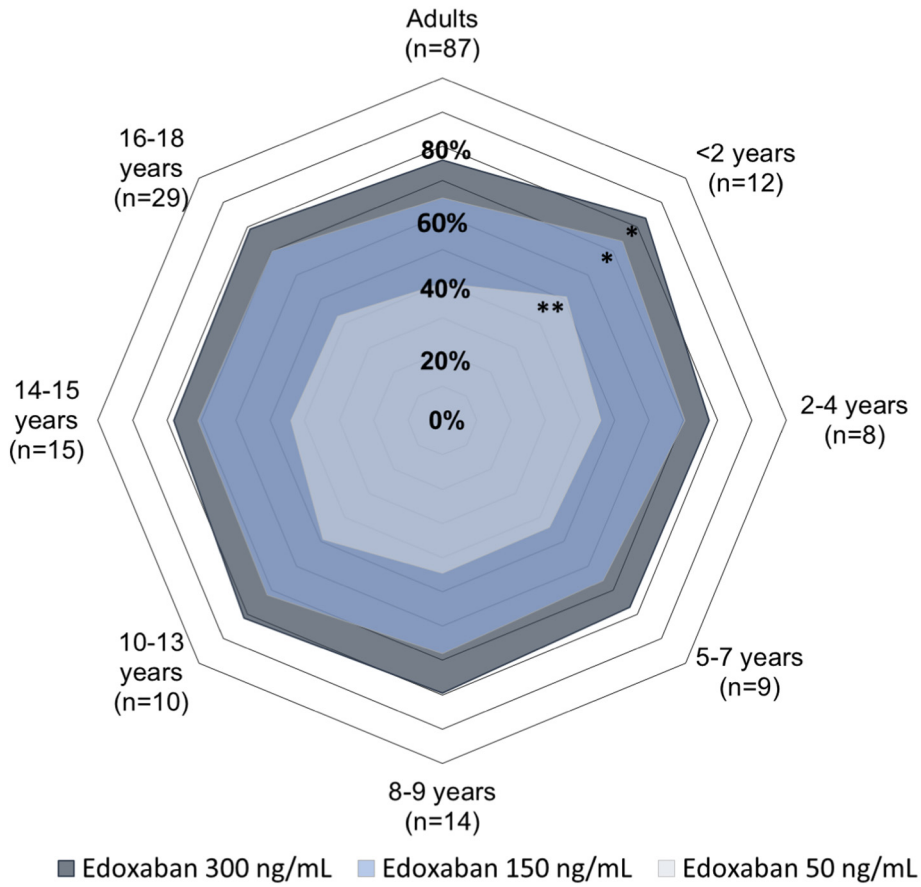

Fig. 5. Percentage of thrombin peak inhibition by increasing edoxaban concentration in the different age groups.
Radar chart showing the more pronounced inhibition of thrombin peak by edoxaban in the < 2 year-age group compared with adults.
$^{**}p < 0.01$, $^{*}p < 0.05$ compared with adults.

et al. [37] showed that in most methods used to measure ETP, the substrates could be cleaved also by alpha2-macroglobulin-bound thrombin, which is inactive *in vivo*, and this could induce a bias in the comparison of adults and children. To overcome this limitation we used the method described by Hemker in which the alpha2-macroglobulin-bound thrombin fraction is estimated and subtracted from the signal [38]. Only children and adults not requiring anticoagulant therapy were included in our study. Edoxaban effect should also be studied in patients with potentially prothrombotic disorders who are likely to receive anticoagulant therapy. An *in vivo* clinical study is needed to evaluate edoxaban efficacy and safety, particularly in children younger than 2 years. The number of samples for the different age groups, especially for the youngest children, was small. This is explained by the limited availability of volunteers who matched the inclusion criteria (healthy subject, without anticoagulant therapy), and the difficulty of obtaining blood samples from young children and in sufficient volume for the different tests. Nevertheless, edoxaban response was different in < 2-yar-old children and it is known that coagulation changes are more important close to birth. A study specifically on this age group would be interesting.

Edoxaban anti-factor Xa activity accurately reflects plasma levels in both children and adults, and its *in vitro* effects are similar at all ages, with the exception of children younger than 2 years of age in whom the anticoagulant effect is increased.

Declaration of interest

None.

Acknowledgements

Authors sincerely thank the technical staff of the Hemostasis Department (University hospital of Clermont-Ferrand) and Elisabetta Andermarcher for English editing.

This study was funded by a grant from Octapharma, Switzerland.

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