Long-term safety and efficacy data on childhood venous thrombosis treated with a low molecular weight heparin: an open-label pilot study of once-daily versus twice-daily enoxaparin administration

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In this open label pilot safety study 80 children over 3 months old with deep venous thrombosis were treated with enoxaparin with a target 4 h anti-factor Xa activity between 0.5-0.8 IU/mL. The children were stratified to receive once daily or twice daily doses. The study end-points were post-thrombotic syndrome, re-thrombosis, bleeding, and therapy-related death. The median duration of treatment was 5 months and the median follow-up was 24 months. No significant differences were found between the two groups of patients. No bleeding or therapy-related deaths occurred. These safety and efficacy data may serve as a basis to initiate an international multicenter study on enoxaparin treatment.

Key words: enoxaparin, pediatric thrombosis, safety and efficacy.

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Clinical studies in adults have demonstrated several benefits of low-molecular-weight heparins (LMWH) over unfractionated heparin (UFH). LMWH are at least as effective as UFH, are associated with a significantly lower frequency of bleeding complications and heparin-induced thrombocytopenia and, since the pharmacokinetics of LMWH are more predictable than those of UFH, the frequency of monitoring via anti-factor Xa assays is minimized.[1] Pharmacokinetic studies in children have indicated that the LMWH enoxaparin can be administered subcutaneously once or twice daily.[2-7] It was recently demonstrated that once daily or twice daily enoxaparin administration had no impact on safety or efficacy rates in the treatment of acute venous thrombosis (DVT) in adults.[8,9] However, it is still unclear whether enoxaparin should be administered once daily or twice daily in pediatric patients with DVT. To obtain more safety and efficacy data in children with DVT prior to a randomized multicenter study, the present open-label pilot study was conducted to compare once daily versus twice daily enoxaparin administration in infants, children and adolescents treated according to a German treatment protocol.[10]

Design and Methods

The present study was performed in accordance with the ethical standards laid down in the updated version of the Declaration of Helsinki Informed, signed consent was obtained from the parents of each study participant.

Study design and study endpoints

This two-center study was an open-label, non-randomized, prospective follow-up study in pediatric patients with a first DVT treated with enoxaparin. The prospectively defined efficacy end-points of the study were post-thrombotic syndrome, recurrent DVT, and patency rates; safety end-points were defined as minor and major bleeding while on LMWH therapy.

Patients and inclusion criteria

Eighty unselected pediatric patients with symptomatic DVT undergoing treatment with the LMWH enoxaparin and consecutively enrolled between January 2001 and December 2004 were analyzed. Inclusion criteria were a venous thrombotic event confirmed objectively by standard imaging methods, i.e. compression sonography, venography, computed tomography (CT), spiral CT or magnet resonance imaging (MRI) and perfusion lung scans, for the diagnosis of DVT and/or pulmonary embolism. DVT/recurrent DVT in the deep veins of the leg was defined when venography performed in the acute phase of a new vascular accident showed fresh thrombotic material within the lumen of a vein, i.e. a new intraluminal filling defect compared with previous tests. Patients younger than 3 months and older than 18 years at the onset of their first DVT were not enrolled. In addition, children who had suffered arterial

thrombosis or stroke were excluded. Further exclusion criteria were premature birth (<36 weeks of gestational age), and ongoing liver or renal diseases.

Adapting criteria from adults, post-thrombotic syndrome was defined by objective signs, e.g. an increase in calf or ankle circumference by 2-4 cm, dark pigmentation of the skin, venous telangectasia, varicose veins or open ulcer.[11,12] Thrombus extension in children with leg thrombosis was defined with respect to the distribution of the initial thrombotic material within the affected vessels: distal DVT < proximal DVT/cerebral venous thrombosis (CVT) < pelvic DVT < proximal and pelvic DVT. Body mass index (BMI) calculated as kg/m^2 was also documented. In this study the presence of post-thrombotic syndrome was evaluated 6-12 months after the acute event.

Anticoagulation protocol

Pediatric patients with a first DVT received enoxaparin 1 mg/kg twice daily during the period of acute thrombosis, with the aim of establishing a 2-4-hour anti-factor Xa activity of 0.6-1.0 IU/mL).[2,5] Following the acute treatment period (days 7-14), LMWH was administered at a standard prophylactic dose once daily (starting dose 1.5 mg/kg/day)[4] or twice daily (starting dose 1 mg/kg twice daily)[2,5] according to the off-label use of enoxaparin in the German treatment protocol.[10] Based on pharmacokinetic data on LMWH in childen,[13] which were similar to those in adults, and bearing in mind the known limitations of the laboratory monitoring of UFH and LMWH therapy[14] no separate pharmacokinetic study was performed for the 1.5 mg/kg/day enoxaparin dosage. In both treatment arms the predefined target anti-factor Xa activities were 0.5-0.8 IU/mL for the 4-hour determination, and >0.1 IU/mL for the 12-hour measurement. Since the German LMWH protocol applied since 1992 has historically favored once-daily administration of LMWH for the management of DVT beyond the acute thrombotic phase, the enrol-lment into the two groups was designed to follow a two to one stratification, performed within the first 3 days after hospital admission: each third patient was treated with LMWH twice daily.

Clinical follow-up procedures

The diagnostic tests and assessments, including physical examination by an experienced pediatrician, were carried out at the onset of thrombosis and 6-8 weeks and 6 months later. Vessel patency was classified as complete, partial or unchanged following radiological evaluation. Complete patency was defined as no clot present in the previously affected vessel; partial patency denoted that a clot inside the vessel lumen had decreased or shrunk, and patency was unchanged if the thrombus remained confined to the same lumen. In addition, worsening was reported when the clot extended to previously unaffected segments. After withdrawal of antithrombotic therapy, asymptomatic pediatric patients enrolled were re-evaluated every 3 months in the first year and at 6-month intervals following the second year.

Blood samples

During the acute treatment period blood samples were collected daily. When prophylaxis was started blood samples were taken at the time of the second and fourth LMWH injections; the dose was adjusted if necessary. When the target range had been achieved further blood samples were taken monthly for asessement of antiXa activity, PTT, D-dimer and blood cell counts. Blood samples were collected 4 hrs/12hrs after LMWH administration by peripheral venipuncture into plastic tubes containing 1/10 by volume of 0.106 mol/L trisodium citrate (Sarstedt) and placed immediately on melting ice. Platelet-poor plasma was prepared within 20 minutes after drawing the blood by 2 x centrifugation at 3000 g for 20 minutes at 4° C; the samples were aliquoted in polystyrene tubes, stored at -70° C and thawed immediately before assaying.

Laboratory analyses

PTT, antithrombin and anti-factor Xa activity were measured on a Dade Behring BCS analyzer using Pathromtin SL™ and BC thrombin-reagent™ from Dade Behring and chromogenic substrates (Berichrom antithrombin™: Dade Behring; coamatic heparin™: Haemochrom). The intrassay coefficient of variation for heparin was 3.6% (0.7 IU/mL), whereas the run-to-run coefficient of variation was 2.8%.

Statistical analyses

Statistical analyses were performed with the StatView 5 software package (SAS Institute). Outcome variables, e.g. number of patients with post-thrombotic syndrome at 6-12 months, recurrent DVT, 6-12 months patency rates, and number of bleeding events were calculated in both groups. Descriptive statistics were applied and results compared by χ^2-analysis or by Fisher's exact test, if necessary. For variables with a non-Gaussian frequency distribution, data are presented as medians and ranges. Evaluations and comparisons between patients of both groups were conducted using the Mann-Whitney test. The level of statistical significance was set at 0.05.

Results and Discussion

Patients

Eighty children (36 females and 44 males) consecutively recruited from the in- and outpatient departments, were enrolled. The median follow-up time was

Table 1. Characteristics and outcome of patients.

	Twice daily group n=30	*Once daily group n=50*	*p value*
Demographics			
age (median and range): years	8.1 (0.3 to ≤18)	8.6 (0.3-17.5)	0.62
body mass index (kg/m^2)	17.1 (11.3-28)	18 (11-40)	0.35
gender (male)	57%	48%	0.47
ethnicity	100% white	100% white	–
Thrombus extension			
distal DVT <	2	1	0.49
proximal DVT <<	14	31	
pelvic DVT/RVT <<<	6	8	
proximal and pelvic DVT	8	10	
LMWH dose (mg/kg):			
Acute therapy		1.0 twice daily	
12-hour anti factor Xa (IU/mL) prior to first prophylaxis dose		0.36 (0.22-0.75)	
duration: acute therapy (days)		7 (5-13)	
LMWH dose (mg/kg/day):			
Prophylactic starting dose	2	1.5	
anti factor Xa level adjusted	1.2 (0.7-3)	1.0 (0.5-2.2)	0.27
4-hour anti factor Xa (IU/mL)	0.57 (0.2-1.0)	0.54 (0.18-0.81)	0.27
12-hour anti factor Xa (IU/mL)	0.12 (0.05-0.3) → n=12	0.17 (0.05-0.32) → n=8	0.66
duration: prophylactic LMWH (months)	4 (1-10)	5 (1-13)	0.25
Efficacy end-points			
post-thrombotic syndrome	2/30	3/50	0.72
rethrombosis	2/30	2/50	0.64
Safety end-points			
bleeding	2/30	1/50	0.64
death	–	–	–

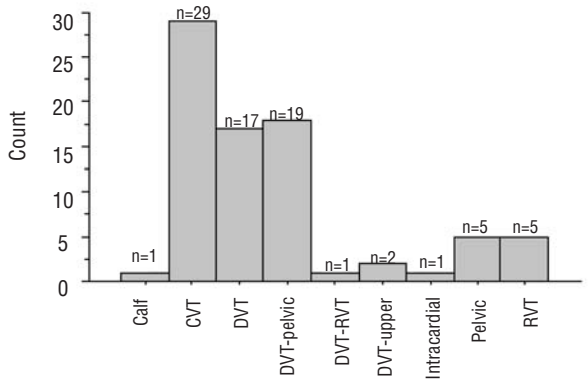

Figure 1. Locations of venous thromboses (DVT) in the cohort investigated (CVT: cerebral venous thrombosis; DVT-upper: DVT located in the upper venous system; RVT: renal venous thrombosis).

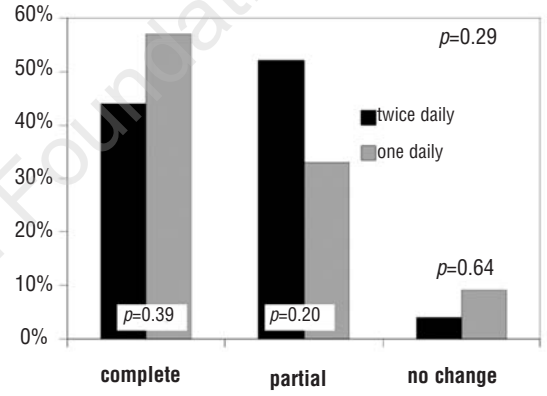

Figure 2. Patency rates in the groups stratified to receive enoxaparin once daily or twice daily. *p*-values are shown for all patency outcomes (complete, partial, no change), and were additionally calculated for the entire model (right upper corner).

24 months (range 12-60). The patients' characteristics are shown in detail in Table 1. The locations of the thromboses are represented in Figure 1. Eighty-five percent of the patients suffered from additional underlying medical conditions. Based on radiological criteria, there was no statistical difference with respect to the severity of DVT, the initial extent of the thrombosis, or underlying medical conditions between the two groups treated with the different enoxaparin regimens (p=0.5).

Prophylactic LMWH therapy was started immediately after the acute treatment period. The target anti-factor Xa activity range along with normal age-dependent values for PTT, antithrombin and D-dimer (*data not shown*) were reached after at least three doses of LMWH in the majority of the children enrolled. The dosage had to be reduced in one 17-year old male receiving twice daily enoxaparin (4-hour anti-factor Xa activity: 1.0 IU/mL to 0.75 IU/mL), and *vice versa* had to be increased in two adolescent girls, one treated once daily (4-hour anti-factor Xa activity: 0.20 IU/mL to 0.4 IU/mL), the other treated twice daily (4-hour anti-factor Xa activity: 0.18 IU/mL to 0.5 IU/mL).

Study end-points

During the follow-up period 6.3% of the children developed post-thrombotic syndrome and recurrent DVT was noted in two cases in each group. Comparable patency rates were documented in both LMWH groups 6 to 12 months after the acute thrombosis (Figure 2). As regards the safety end-points, the number of bleeding events was similar in both treatment groups. Minor bleeding occurred twice in the group receiving enoxaparin twice daily: the 17-year old male with an anti-factor Xa activity of 1.0 IU/mL suffered an ankle joint bleeding, and a minor subdural bleed occurred in a patient with cerebral venous sinus thrombosis. In addition, there was an episode of minor prolonged bleeding from a puncture site in one child treated once daily with LMWH. Neither heparin-induced thrombocytopenia (platelets <120,000/μL) nor therapy-

related deaths were observed during either the treatment or follow-up period.

Until recently, UFH followed by oral anticoagulation has been considered the initial anticoagulant regimen of choice for the treatment of DVT in children.[15] It has, however, now been demonstrated that therapy with LMWH is equally efficacious and safe as UFH in the prevention and treatment of DVT in adult patients.[1, 4,16] In addition, it has been stated that once daily administration of LMWH in adult patients with DVT is similarly efficacious and safe compared as twice daily administration.[9] These data were first reported for the LMWH enoxaparin by Merli *et al.*, who administered fixed doses of 1.0 mg/kg body weight twice daily or 1.5 mg/kg once daily.[8]

Drawing on the study by Merli *et al.*, the present survey in infants and children aged >3 months to ≤18 years with acute DVT was conducted as a pilot study in 2001. Like Merli *et al.*, we found no significant differences between once daily and twice daily prophylactic enoxaparin administration with respect to the defined efficacy and safety endpoints. Bearing in mind the study limitations, e.g. a non-randomized study design and an underpowered pediatric study cohort, the results presented here in children with DVT, which are in line with results obtained from adults, encourage us to initiate a multicenter, randomized trial.

RS and UN-G were responsible for the conception of the study, patient enrollment, the integrity of the study, analysis of the data, and writing the manuscript; CD, CB and NM were responsible for sample collection and data documentation, and AH was responsible for the statistical work-up.

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