Reduction of painful vaso-occlusive crisis of sickle cell anaemia by tinzaparin in a double-blind randomized trial

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Summary

A randomized double-blind clinical trial was performed to test the safety and efficacy of a low-molecular-weight heparin, tinzaparin (Innohep®), for the management of acute painful vaso-occlusive crisis characteristic of sickle cell anemia (SCA). We studied 253 patients with acute painful crisis but with no other complications of SCA, randomized to treatment or control groups. In the treatment group, 127 patients received tinzaparin at 175 IU/kg, subcutaneous once daily, along with supportive care including morphine analgesia; in the control group, 126 patients received placebo and the same supportive care. The maximal experimental treatment period was seven days. Analysis revealed a statistically significant reduction in number of days with the severest pain score, overall duration of painful crisis, and duration of hospitalization ($p < 0.05$ for each comparison of tinzaparin vs. placebo). The decline in pain intensity was sharper for tinzaparin-treated patients, and complications consisted of two minor bleeding events that were reported and treated by cessation of tinzaparin. This investigation demonstrated that tinzaparin, administered at its approved treatment regimen, reduced the severity and duration of acute crisis of SCA.

Keywords

Sickle cell anemia, tinzaparin, heparin, pain, genetic disease

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Introduction

Sickle cell disease is a systemic disorder that is caused by a mutation (Glu6Val) in the gene that encodes β globin. The sickle hemoglobin molecule (HbS) is a tetramer of two α-globin chains and two sickle β-globin chains, and it has the tendency to polymerize when deoxygenated. HbS facilitates abnormal interactions between the sickle erythrocyte and leukocytes and endothelial cells, which trigger a complex pathobiology. This multifaceted pathophysiology provides the opportunity to interrupt the disease at multiple sites, including polymerization of HbS, erythrocyte density, and cell-cell interactions. For example, it is possible to induce higher concentrations of fetal hemoglobin, which disrupts the pathology-initiating step of HbS polymerization. Furthermore, it is possible to improve the hydration of sickle erythrocytes, and it might be feasible to counteract the endothelial, inflammatory and oxidative abnormalities of sickle cell disease. A therapeutic approach that targets several sites of pathobiology might be most promising (1).

The clinical hallmark of this disease is the painful acute "crisis" that has, in spite of therapeutic advances, continued to be a treatment challenge. Such crises occur with variable frequency and duration, and they commonly require hospitalization. Intervention capable of ameliorating crises of sickle cell anemia (SCA) might be expected to improve the overall natural history of the disease. Because of the multiple factors involved in SCA, we hypothesized that an agent with polypharmacological actions, such as heparin or low-molecular-weight heparins (LMWHs), might have potential impact on the disease process.

Preliminary evidence suggests that the heparins, which have anti-adhesive as well as anti-thrombotic and anti-inflammatory properties, may be worthy of further testing for effects on SCA (3–5). The LMWHs that have become available during the past decade are particularly appealing for clinical trials because of the safety profile in standard approved doses and because they can be self-administered in the outpatient setting in areas of the world in which SCA is prevalent. Because of the favorable pharmacokinetic profile of tinzaparin as once-a-day LMWH (5) and its potentially favorable pharmacodynamic effects on various

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cellular events including endothelial tissue factor pathway inhibitor (5), tumor necrosis factor-α (4, 5), nitric oxide (NO) modulation (3, 5), and P-selectin effect (6) in addition to its anticoagulant effects, the use of LMWH tinzaparin was justified in the treatment of acute painful crisis of SCA.

Materials and methods

This prospective, randomized, double-blind clinical trial studied 253 patients admitted to the hospital (King Abdulaziz University Hospital, King Fahd General Hospital and King Abdulaziz Oncology Center; Jeddah, Saudi Arabia); the trial was approved by the ethical committee and registered at the concerned centers. The patients were in acute painful crisis of SCA, which was defined as homozygous sickle cell (SS) disease characterized by a single major hemoglobin band in the position of HbS on alkaline and acid hemoglobin electrophoresis, as well as compatible HbA_2 and HbF levels. Where possible, a confirmatory electrophoresis was performed on both parents. Acute crisis was defined as the presence of bone pain with features typical of the painful crisis and not resulting from any other pathology. The pain had to be of sufficient severity to require narcotic analgesia (i.e. pain not relieved by simple analgesics such as acetaminophen).

Patient entry and management

Inclusion criteria

- SCA patients with homozygous sickle cell (SS) disease
- Patients admitted through the emergency room with painful vaso-occlusive crisis† severe enough to require narcotic analgesia
- Age >12 years of either sex

Exclusion criteria

- Presence of medical or surgical contraindication to LMWH
- Pregnancy
- Low platelet counts (<100,000/dl) or impaired hemostasis on admission in the form of International Normalized Ratio (INR) >1.4 or prolonged APTT >5 seconds of the hospital normal range
- Complicated SCA
- History of cerebral vascular accident (CVA)
- Current aplasia
- Acute chest syndrome
- Exchange transfusion
- Sequestration
- Anticoagulants therapy for other etiology
- Patients with painful crises within the month before this admission.
- Women on hormonal contraception

Patients were excluded if they were less than 12 years of age, had other vascular complications of SCA (such as prior stroke, current aplasia, acute chest pain), or a history of painful crisis within the preceding month. Patients were also excluded if they required exchange transfusion, were immobilized, required anticoagulant therapy for any other reason, or had laboratory evidence for impaired hemostasis (such as a platelet count of <100,000, an INR >1.4, or a partial thromboplastin time >5 seconds above the upper limit of normal for the hospital laboratory). Women with SCA were excluded if they were pregnant or using hormonal contraception because of the potential impact of contraceptives on thrombosis and hemostasis (7). Patients were excluded from analysis if they developed any condition while on study that required treatment with any anticoagulant or antiplatelet therapy.

Upon admission to each participating center, all patients underwent routine medical history, physical examination, and laboratory testing. With determination of eligibility by the criteria specified above, informed consent was obtained. All consenting patients entered received the same standard therapy consisting of hydration and analgesia consisting of morphine, 1 mg per hour (h), given intravenously. Tinzaparin and placebo were provided by the manufacturer. All drug supplies were appropriately packaged, labeled, and kept in a locked, safe area under appropriate storage conditions with access limited to persons authorized by the investigator and those who directly involved in the study.

Management plans

Pre-treatment evaluation included the following: History and physical examination, consent, complete blood count, prothrombin time, partial thromboplastin time, liver function test, urea, creatinine and electrolytes.

Management included the following: Standard analgesia therapy: morphine 1 mg/h intravenous infusion and rehydration with normal saline. Patients were randomized consecutively into either the study group where the patient received in addition tinzaparin 175 anti-Xa IU/kg, subcutaneously (s.c.), once-daily for seven days or the control group where the patient received in addition placebo.

Endpoint included the following: i) Clinical improvement and pain reported by the patient as zero degree on the numerical pain scale; ii) Therapy discontinuation after seven days regardless of the outcome; iii) Appearance of any complications including bleeding, HIT, or other complications.

Assessment of outcome: Each case's findings are outlined on a separate form summarizing personal and clinical data, total

Table 1: Characteristics and effect of tinzaparin on painful crisis and duration of hospitalization in sickle cell patients.

	Study group (n = 127)	Control group (n = 126)
Mean age (years)	22.8 ± 4.5	21.6 ± 3.8
Sex		
– Male	58 (46%)	63 (50%)
– Female	69 (54%)	63 (50%)
No. of days with severest pain score on the NMS	1.28 ± 0.20*	1.74 ± 0.15
Duration of painful crisis (days)	2.57 ± 0.45*	4.35 ± 0.78
Total duration of hospitalization (days)	7.08 ± 1.8*	12.06 ± 2.2

Tinzaparin was administered at 175 IU/kg, s.c. once a day for 7 days. Data represent mean ± SD, *P <0.05. NMS = numerical pain scale.

period of hospitalization in days, rate of decline of the pain intensity over the days of the study subjectively assessed and filled by the patient in a separate form using the numerical pain scale (NMS) assessment, the number of days on which the patient experienced the highest intensity on the NMS, the duration of painful crisis in days, i.e. the number of days needed for the pain to decline from the highest score to zero on the NMS, and occurrence of complication during hospitalization

Concomitant medication: The patients were not put on any anticoagulant or antiplatelet agent. Of the 253 patients entered into the study, 127 were randomized to receive tinzaparin and 126 to receive placebo. The recruitment and the duration of the study took a total of 48 months. The data-safety-monitoring board was the ethical committee fully responsible for the approval of the study prior to the implementation. Mean age and gender distribution between study (tinzaparin) and control groups is shown in Table 1. Patients in the treatment group received 175 IU/kg s.c. once daily of tinzaparin, whereas placebo was given similarly to patients in the control group. Treatment with tinzaparin or placebo began upon admission following informed consent and was repeated daily until the end of the study on day 7 regardless of the treatment outcome. Thereafter, patients were treated with standard analgesics and other appropriate conventional management as needed. All patients were closely monitored by the participating specialist. Hematological parameters including platelet counts were monitored as discussed earlier in the management plans.

The *clinical course of study patients* was assessed in the same manner for both treatment groups using the NMS (8). Outcome measures recorded on study data sheets included assessment of the number of days in which the patient experienced the severest degree of pain, the rate at which the pain intensity declined over time, and the duration of painful crisis and overall hospitalization recorded in days. Clinical improvement was defined as achievement of a pain score <2 reported by the patient on the NMS. The overall duration of painful crisis was defined as the number of days needed for the pain to decline from the highest score to <2 on the NMS.

This study was approved by the ethics committee of each participating center and was conducted in concordance with globally accepted standards of good medical practice and in agreement with the Helsinki declaration and local health regulations.

Adverse events (AEs) were recorded in all patients in both treatment groups and transmitted to the data-safety-monitoring board. These included any undesirable event occurring concomitant with the use of the experimental drug, whether drug-related or not, including any side effect, injury, toxicity, or sensitivity reaction. AEs also included development of other vascular complications of SCA, as mentioned in the exclusion criteria, and any undesirable clinical or laboratory change that does not commonly occur in patients with SCA. Complications considered possibly due to experimental therapy observed during this study, such as bleeding and heparin-induced thrombocytopenia (HIT), were also recorded. A complete blood count including platelet count was performed on day 4 of the study to assess development of HIT syndrome. Major hemorrhage was defined as overt hemorrhage associated with at least one of the following: death, the need for a transfusion of at least two units of packed red blood cells or whole blood, or a fall in hemoglobin >2.0 g/l as compared with baseline, or the occurrence of retroperitoneal (confirmed by ultrasonography or computerized tomography) or intracranial bleeding. Minor hemorrhage was defined as bleeding of severity that did not meet the criteria for major hemorrhage. Minor hemorrhages included, but were not limited to, epistaxis lasting >5 minutes or requiring intervention; ecchymosis or hematoma >5 cm; hematuria not associated with urinary catheter, trauma, or infection; or gastrointestinal hemorrhage not related to incubation or nasogastric tube placement. Bleeding at the injection sites was evaluated daily by checking for hematomas >5 cm, considered as minor hemorrhages, or other skin reactions, particularly allergic reactions. Given the safety record of the study drug, the possibility of drug overdose was considered negligible. Patients were allowed to withdraw from the study at any time if they chose to do so. Investigators were at liberty of discontinuing the study drug if, in their judgment, continued treatment was not in the best interest of the patient. In either case, data collection continued until the end of the defined study interval.

Statistical analysis

Information recorded on study data sheets were transferred to IBM cards and processed using an IBM-PC with the Windows operating system "SPSS-V10." Data were analyzed using descriptive statistics including determination of the mean (X), standard deviation (± SD), and range (min-max). Analytical statistical methods used included Student's t-test for comparisons between two independent means, Pearson's correlation coefficient *r* for the relation between two sets of quantitative data, the Chi^2-test for analysis of qualitative data, and calculation of the p-value to test for level of significance. Levels for $p > 0.05$ were considered not significant, $p < 0.05$ significant, and $p < 0.001$ highly significant. Re-analysis of the data was also carried out using ANOVA.

Results

Patients entered to the treatment (n = 127) and control (n = 126) groups in this study were comparable for age (22.8 ± 4.5 vs. 21.6 ± 3.8 years, respectively) and sex (45.7% and 50% male, respectively). Data presented in Table 1 show that tinzaparin-treated patients had significantly fewer total hospital days (mean of 7.08 vs. 12.06 days), overall days of crisis (mean of 2.57 vs. 4.35 days) and days of severest pain score (mean of 1.28 vs. 1.74) compared to placebo-treated patients, respectively. Data shown in Figure 1 show that, while pain scores were comparable between treatment groups at entry, pain declined or resolved more rapidly during the first four days of treatment in the tinzaparin-treated group. Tinzaparin treatment was associated with two minor bleeding events that were reported and treated by cessation of the tinzaparin.

Discussion

This double-blind randomized clinical trial was designed to test the hypothesis that the LMWH tinzaparin in ordinary approved treatment doses would improve the course of SCA in acute crisis because of its efficacy on coagulation and beyond (5). In SCA

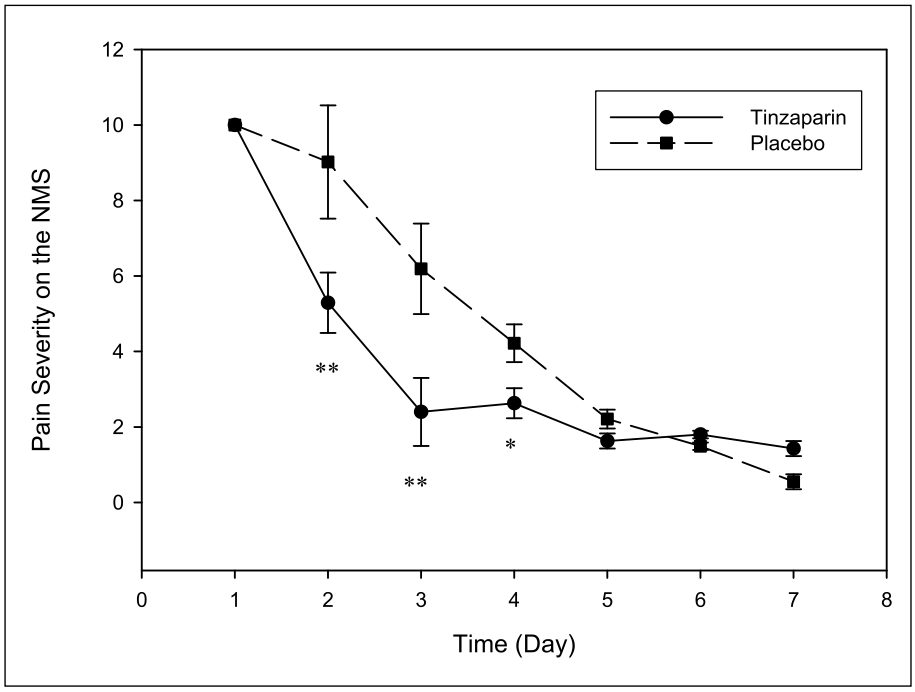

Figure 1: Effect of tinzaparin on pain severity. Seven days' follow-up of pain severity score in the tinzaparin and placebo groups. NMS = numerical pain scale. *p <0.05; **p <0.01 (ANOVA). Tinzaparin resulted in more rapid resolution of pain, and by days 5–7 pain severity scores between placebo and tinzaparin were not statistically different.

patients, there are significant alterations in the various components of hemostasis, including platelet activation, endothelial cell activation, increased procoagulant activity, decreased natural anticoagulants, and imbalanced fibrinolytic system (9–18). These alterations account for the concept that SCA is a hypercoagulable or prothrombotic state, and histopathologic studies have confirmed the role of thrombosis in SCA (19–21). Narrowing of large arteries with superimposed thrombosis is the most common cause of stroke in SCA (22–26). Post-mortem studies in SCA patients with pulmonary disease demonstrate old and new thrombi within the pulmonary vasculature (20). Indirect data also link thrombosis with many other complications of SCA, including avascular necrosis, leg ulcers, and pregnancy complications (27). In a prospective randomized double-blind trial of ticlopidine (250 mg b.d.) or placebo for one month, ticlopidine blocked platelet activation; however, this alone did not improve platelet survival or prevent sickle crisis. In view of evidence of platelet activation in sickle cell disease, however, a longer trial with antiplatelet drugs might be warranted (28, 29). An earlier pilot trial in four subjects with well-documented severe recurrent painful sickle cell crises given long-term mini-dose of unfractionated heparin demonstrated improvement in all four subjects while receiving heparin in terms of duration of hospital stay (30). Ultimately, proof that these mechanisms are in play *in vivo* will require the demonstration of clinical efficacy of therapies based on inhibition of sickle cell rigidity, vascular tone and abnormal cell adhesion (31–33). In fact, hydroxyurea, which reduces the frequency of crises in SCA by increasing expression of fetal hemoglobin, among other effects, also results in downregulation of CD36 and VLA4 expression in sickle red cells (34). This property of hydroxyurea may contribute to its clinical benefit. There is considerable interest in anti-adhesion therapies in SCA, largely because of the potential involvement of this class of adhesion molecules in SCA. The results of clinical trials of such strategies that interfere with the binding site of sickle red cells to the endothelium represent a novel approach (35, 36).

Heparin and LMWH are shown to increase the plasma level of NO. NO, a potent vasodilator that inhibits platelet aggregation, activation, and secretion has also been shown to inhibit sickle red cell adhesion to the endothelium *in vitro* (37). In fact, NO has been given by inhalation to SCA patients with severe acute chest syndrome and produced a dramatic improvement in oxygenation (38). Inhaled NO might have the additional benefit of increasing oxygen affinity in sickle red cells and, therefore, could potentially reduce HbS polymerization (39). It is intriguing that arginine, the substrate for NO production, has been used in non-SCA patients in several studies (40, 41). Arginine, given orally, increases levels of both exhaled and plasma NO, although these effects are transient (42). In addition, endothelial and vasodilatory dysfunction is corrected in hypercholesterolemic patients given L-arginine (43). In contrast, tinzaparin demonstrated a longer-lasting increase in plasma tissue factor pathway inhibitor and NO (3, 44).

Clinically approved heparins have differing abilities to inhibit selectins, likely explained by size distribution. It should be possible to size fractionated heparins and inhibit selectins at concentrations that do not have a large effect on coagulation. Caution is also raised about the current preference for smaller heparins. Despite equivalent anticoagulation, hitherto unsuspected benefits of selectin inhibition in various clinical circumstances may be unwittingly discarded. Tinzaparin demonstrated greater potency in inhibiting P-selectin as compared to other LMWH (45). In that regard, sickle cells were shown to adhere to immobilized recombinant P-selectin under flow conditions. This adhesion was inhibited by heparin, in a concentration range that is clinically attainable. These findings and the general role of P-selectin in initiating adhesion of blood cells to the endothelium suggest that heparin may be useful in preventing painful vascular

occlusion (45). Stevenson et al. (6) showed that one (tinzaparin) of three LMWHs showed increased selectin inhibitory activity, and the synthetic pentasaccharide, fondaparinux, showed none when normalized to anticoagulant activity.

In conclusion, the present clinical trial assessed the efficacy and safety of LMWH tinzaparin therapy in the management of acute painful vaso-occlusive crisis. The observed benefits with tinzaparin support further studies to define optimal dose and schedule aimed at both treatment and prevention of sickle crisis.

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