AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 33 NUMBER 4 DECEMBER 2004

ISSN 1116—4077

Afr. J. Med. med. Sci. (2004) 33, 335-340

Efficacy and safety of enoxaparin, a low molecular weight heparin in the prevention of deep vein thrombosis in Nigerian patients after orthopaedic surgery

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Summary

The objective was to determine the efficacy and safety of Enoxaparin as an antithrombotic agent in orthopaedic patients at risk for thromboembolism. 49 patients who had lower limb orthopaedic surgery were studied. They received subcutaneous Enoxaparin 40mg 12 hours before surgery and subsequently, daily for one week. Blood specimens were drawn at 2 and 12 hours after the first injection, and 24 hours after the fourth injection for anti Factor Xa assay. Specimens were also taken preoperatively, 1st, 5th and 7th post operative days (POD) for determination of Packed Cell Volume (PCV), Haemoglobin level, White Blood Cell (WBC) and Platelet Counts. The mean pre-treatment, 2, 12 and 24 hours anti Factor Xa clotting times were 14.5 ± 0.8, 36.2 ± 5.6, 30.6 ± 9.8 and 25.8 ± 9.3 seconds respectively. The changes were significant, $P = 8.2 \times 10^{-12}$. The 2 and 24 hours clotting times corresponded to plasma heparin concentration level of 0.12 – 0.22U/ml read off from prepared Enoxaparin standardisation curve. Significant changes were observed in haemoglobin level, PCV, WBC and Platelet Counts when preoperative, 1st, 5th and 7th POD mean values were compared by Analysis of Variance – $P<0.01$ in all cases. The study showed that Enoxaparin 40mg daily caused hypocoagulation within prophylactic range of 0.12 – 0.22U/ml of heparin in the plasma. Changes in blood counts were within the limits expected post surgery.

Keywords: *Efficacy, DVT, orthopaedic exioxaparin*

Résumé

Le but de cette étude de l'efficacité et la protection de l'énoxaparine , un antithrombotique aux patients orthopédique a risque de thromboembolisme. 49 patients ayant eu une chirugie de la jambe étaient étudiés. Ils ont recu l'énoxaparine subcutanée de 40mg a 12 heures avant la chirugie et successivement par jour pour une semaine. Les échantillons de sang étaient collectés a 2h, 12 heures aprés la premiére injection du médicament et à 24 heures aprés la 4 ième injection pour le facteur anticoagulant Xa et ensuite analysés. Les échantillons préoperatives a 1ier, 5ième and 7ième jour post chirugie aider à déterminer l'hématocrite , le taux d'hémoglobine, le nombre des globules et platelets sanguine. La moyenne du pré-traitement à la 2 ième , 12 et 24 ième heures du facteur anticoagulant Xa étaient 145 ±*0.8, 36.2*±5.6, 30.6±9.8 et 25.8±9.3 seconde respectivement. La 2 ième et 24 ième heures de coagulation correspondait a la concentration de l'héparine en plasma de 0.12-0.22ul/ml de la courbe de standardisation. Alors l'anoxaparine à 40 mg par jour cause l'hypocoagulation dans les limites prophylatiques d'héparine dans le plasma et un niveau de santé post chirugie normale. Des changements significatif étaient observés sur le taux d'hémoglobine, PCV, RBCs et platelets quant la pré-operation de 1ième , 5ième, 7 ième POD valeur moyenne chez tous les cas. Les changements de paramétres hématologiques étaient entre les limites connues aprés la chirugie.

Introduction

Major orthopaedic surgery particularly of the lower limbs places the patient in a high risk for venous thromboembolism. For those patients undergoing hip surgery or knee reconstruction, the risks of deep venous thrombosis (DVT) vary between 15 and 75% [1]. The higher risk being associated more often with hip surgery possibly because of damage (during surgery) to the venous wall of the complex anastomoses around the hip [2]. This thromboembolic phenomenon, though largely preventable, is often clinically silent and not recognised until complications set in. Clinical diagnosis is undependable and serial surveillance of all high-risk patients is either invasive (Venography) or expensive (Doppler Ultrasonography or Impendence Plethysmography) [3].

The most feared of complication of venous thrombosis is pulmonary embolism. This is because two-thirds of patients who succumb to fatal pulmonary embolism die within 30 minutes after the onset of embolisation, a time too short for instituted therapeutic intervention (if available) to be effective [4]. This rapidity of death may explain the observation that fatal pulmonary embolism is the most common *preventable cause* of

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hospital deaths [5]. For maximal reduction in mortality from pulmonary embolism therefore, the approach should be a prophylactic track rather than relying on treatment of diagnosed thromboembolism [6]. As such, prophylaxis for patients at risk using standard heparin or its low molecular weight derivatives is recommended by most authorities [1, 7-9].

Hitherto, prophylaxis for venous thromboembolism involves the use of either unfractionated heparin given intravenously or the use of low-dose aspirin. Practicing Physicians in Nigeria are still unfamiliar with the use of low molecular weight heparin which is now in common use as a prophylactic agent for prevention of venous thromboembolism in the developed world. In the present communication, we studied the effectiveness of a low molecular weight heparin (Enoxaparin) as a safe prophylactic antithrombotic agent by determining its ability to induce hypocoagulable state without precipitating excessive haemorrhage in Nigerians undergoing orthopaedic surgery. To our knowledge, this is the first report of the use of low molecular weight heparin as a prophylactic agent for the prevention of thromboembolism in Nigerians.

Subjects and methods of study

Subjects

Subjects aged between 17 and 90 years who require major orthopaedic surgery of the hip or knee and whose surgery require general anaesthesia were recruited after informed consent was obtained. Exclusion Criteria were patients on any anticoagulant or antiplatelet therapy, those with documented history of bleeding disorders, chronic renal failure, history of hypersensitivity to heparin, pregnancy or lactation and the need for any intramuscular injection during the period of study. The study was approved by the Ethical Committee of the Lagos University Teaching Hospital.

Methods

Enoxaparin 40mg/0.4ml was administered subcutaneously according to the following protocol: a fold of skin in the anterior abdominal wall was held between the thumb and the index finger. The entire length of the needle of the pre-filled syringe was gently inserted perpendicularly into the subcutaneous tissue. The 0.4ml content of the syringe was injected wholly. Injections were given 12 hours pre-operatively, the evening after the operation and on daily basis for subsequent six (6) days [a total of eight injections].

The drug therapy was monitored for –

A. *Efficacy:* by determining hypocoagulable effect of Enoxaparin on patients' plasma using anti Factor Xa assay. Patients' plasma for this test was sampled at 2 hours, 12 hours after administration and on the 5th day just before the administration of the 5th day dose (approximately 24 hours after the 4th day dosing).

B. *Safety:* by determining changes in some haematological parameters – Packed Cell Volume, Haemoglobin, Platelet Count, WBC Count on the 1st, 5th and 7th post-operative days. Clinical assessment for evidence of bleeding, i.e. pain at site of injection, frank bleeding and haematoma.

Anti-Activated Factor Xa Assay

The Low Molecular Weight Heparin (LMWH) preferentially inhibits Factor Xa more than it does Factor IIa (i.e. thrombin). It is therefore more appropriate to use the inhibitory effect of LMWH on Factor Xa as a measure of the level of heparin in the blood[10]. The higher the plasma concentration of heparin, the more is inhibition of Factor Xa and the longer is the clotting time.

A standard curve (fig. 1) relating clotting time in seconds to heparin concentrations in units/ml was prepared by carrying out clotting time tests on standard heparinised pooled plasmas with known concentrations of heparin. The trial drug was used to prepare the standard heparinised plasmas as detailed in the manufacturer's manual of Accuclot-Heptest® - H1 kit supplied by Sigma Diagnostic; (catalog number CRS114).

The clotting time was performed by adding 10ml of commercially prepared activated factor X to 100ml of standard plasma or the test plasma in a pre-warmed (37°) test tube. The mixture is incubated for exactly 120 seconds. During the period of incubation, heparin in the test or standard plasma inhibits the added Factor Xa.

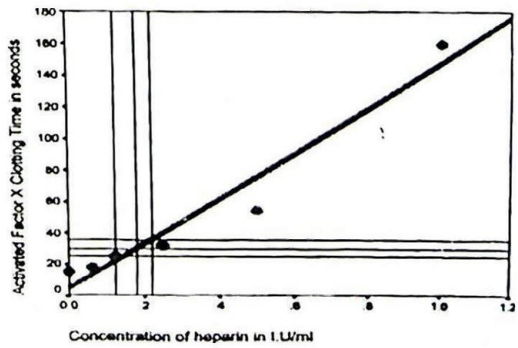

Fig. 1. Standardisation curve of enoxaparin

The plasma with high concentrations of heparin will inhibit larger proportion of the added activated factor X such that after recalcification (using a recalmix at the end of 120 seconds), little amount of Factor Xa is left in the mixture to form prothrominase complex required to convert prothrombin to thrombin, which in turn converts fibrinogen to fibrin. Therefore, the clotting time (the time between recalcification and appearance of clot) will be prolonged. The reverse is true for plasma with low concentrations of heparin.

The heparin concentration of the test plasmas corresponding to their respectively clotting times are interpolated from the prepared standard curve. Full blood count was carried out according to standard method as described by Dacie & Lewis [10].

Statistical Analysis: Changes in the PCV, Haemoglobin Concentration and Platelet Count pre-operatively and on days 1, 5 and 7 post-operatively were compared using the student t test and Analysis of Variance. Taking effective prophylactic range of plasma heparin level to be 0.05 – 0.2u/ml [10], number of subjects having values within and outside this range after dosing was determined.

Table 1: Age and sex distribution of 47 trial subjects

Age Group	Male	Female
≤19	2	0
20 - 29	12	3
30 – 39	6	1
40 – 49	5	4
50 – 59	4	2
60 – 69	2	4
70 – 79	1	1
Total	32	15

**Age of two subjects was not documented.*

Table 2: List of types of orthopaedic surgery amongst the trial subjects

Type of Operation	Number of Patients	percentage
Open Reduction and Internal Fixation	26	53.1
Austin-Moore Arthroplasty	7	14.3
Arthrodesis	6	12.2
Patelloplasty	3	6.1
Bone Grafting	1	2.0
Osteotomy	1	2.0
Sequestrectomy	1	2.0
Tumour Excision	1	2.0
Others – not stated	3	6.1

Results

Demographic data

A total of 49 patients were enrolled into the study. Of these, there were 16 females and the rest were males. The females tended to be older patients with mean age (±SD) being 49.3 ± 18.2 as compared with the male values of 36.4 ± 14.11, P<0.02 (Table 1).

Risk factors

The commonest risk factor for DVT prophylaxis was immobilisation in 100% of the cases while 10 (20% of patients had additional risk factors viz. increased age, surgery for malignancy, diabetes and cardiac problems. Significant percentage (53%) of the trial subjects required Open Reduction with Internal Fixation (ORIF) indicated mainly for fractures either of femoral head, shaft of femur or tibia (Table 2).

Table 3: Changes in anti Factor Xa Clotting Time over a 24 hour period after enoxaparin administration

Time after Enoxaparin dosing	n	Mean Time in Sec	Std	F-statistics Comparing all the mean time	P-Value Comparing all the mean time	Number of subjects with heparin conc <0.05IU/ml
Pre-treatment	20	15.4	0.8			20
2 Hours	17	36.2	5.7			0
12 Hours	19	30.6	9.8	27.2	8.2×10^{-12}	0
Before 5th day dosing (i.e. 24 hours after 4th dosing)	18	25.8	9.3			0

Measure of efficacy

Twenty of the 49 subjects were studied for the ability of the Enoxaparin to induce hypocoagulable state. The anti-factor Xa activity (heparin level) in the pretreatment plasma served as the control. The clotting times for the pretreatment plasma varied between 15 and 18 seconds with a mean (±SD) of 14.5 ± 0.8 seconds. Two hours after Enoxaparin administration, the clotting time increased to a mean value of 36.2 ± 5.6 seconds. At 12 hours, factor Xa clotting time was 30.6 ± 9.8. The 5th day value which represented 24-hour value was 25.8 ± 9.3 seconds. The change in mean values was significant, F-statistics = 27.2, $P = 8.2 \times 10^{-12}$ (table 3). The prolonged clotting time after Enoxaparin administration corresponds to mean low molecular weight heparin levels of 0.22, 0.18 and 0.125u/ml at 2 hours, 12 hours and 24 hours post therapy respectively. These values were read off from Enoxaparin standardisation curve (fig. 1). None of the subjects have values outside prophylactic range (i.e heparin concentration lower than 0.05u/ml at 2 hours, 12 hours

Table 4: Post operative changes in the mean (±SD) of some haematological values over a week period.

	PCV% n = 49	HB g/dl n = 47	WBC x 10⁹/L n = 22	Platelet Count x 10⁹/L n = 49
Pre-study	37.6 (10.3)	12.8 (3.07)	6.1 (2.4)	237.6(75)
1st POD	33.6 (5.5)	11.2 (1.9)	8.3 (3.4)	186.2 (113.0)
				186.3
5th POD	30 (4.8)	9.63 (1.7)	7.3 (3.1)	225 (63)
7th POD	30.7 (4.7)	9.9 (1.7)	9.27 (4.2)	276.4 (93)
				276.5
F-statistics	11.72	18.72	3.48	6.34
P-Value	<0.01	<0.001	<0.05	<0.01

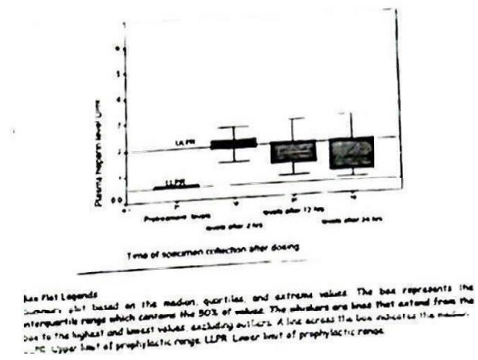

Fig: 2: Median, quartile and extreme values of enoxaparin after daily dosing

Table 5: List of concomitant therapies for patients who had orthopaedic surgery and received subcutaneous Enoxaparin 40mg dly for one week.

Surgery Related Therapies				Specific Therapies			
Antibiotics		Analgesics		Antihypertensives		Others	
Name	n	Name	n	Name	n	Name	n
Rocephin	14	Paracetamol	4	Aldomet	2	Soluble Insulin	2
Zinacef	10	Analgin	3	Regroton	2	Folate	1
Zinnat	8	Tramal	3	Brinerdin	1	Iron tablets	1
Ampiclox	6	Pentazozine	1	Moduretic	1	Maloxin	1
Flagyl	5	Unspecified	3				
Genticin	2						
Ciproxin	1						
Claforan	1						
Fortum	1						
Unspecified	1						

Table 6: List of adverse events.

Event	Frequency	Percentage (%)	Possible Relationship of Event
to Trial Drug			
Leg swelling	2	4	No
Excessive sweating	1	2	Yes
Haematoma	1	2	Yes
Major bleeding	4	8	No

and 24 hours post dosing respectively. Table 3 and (Figure 2).

Measures of Safety

Full Blood Count (Table 4)

PCV and Hb levels fell significantly during study period. P<0.01. The platelet count fell immediately post surgery but rose significantly by the 7th post operative day. – P<0.01. The total WBC count also increased significantly – F = 3.48, P<0.05.

Concomitant medication

All patients except one received antibiotics and, 9 out of 49, also received analgesic either orally or intravenously. List of concomitant therapies is shown in table 5. Hypertensive (2) and diabetic patients (2) also continued on their usual medications – Brinerdin, Regroton, Aldomet and soluble insulin. A patient with sickle cell anaemia continued on his routine folate therapy.

Clinical outcome

41 of 49 (83.7%) subjects completed the trial with no untoward effects. The commonest observed side effect was bleeding in 8% of the patients, which required transfusion of a total of 28 units of blood. One of the

bleeding episodes was however expected as the patient presented with a vascular tumor that is notable for excessive bleeding. This patient had epitheliod haemangio pericytoma of the right femur. Alone, he received 11 units (39%) of the total blood transfused. Only one of 49 (2%) patients developed distal deep vein thrombosis diagnosed clinically but not proven by diagnostic investigative procedures, e.g. leg scanning with I [125] labeled fibrinogen, Doppler Ultrasound or Venography. Other observed adverse effects are noted in Table 6.

Discussion

A total of 49 patients were recruited into the study, 16 females and 33 males. The females tended to be older patients who had surgeries related to fracture neck of femur and osteoarthritis. There was a significant difference in the ages of these females compared to the age group of men who had surgery for various fractures of the lower limb. This finding suggests that young males are more likely to suffer traumatic fracture and elderly females, pathological fracture.

There were statistically significant changes in the levels of haemoglobin, platelet and white cell counts between the pre-operative values and the values obtained in the 1st, 5th and the 7th post-operative days. The change in the haemoglobin value may be attributed to haemorrhage during surgery, as a blood loss of more than 8% is not unexpected for most major limb surgery [11].

The initial fall in platelet count may be the result of the consumption of platelets in primary haemostasis at the site of surgery [12]. Also, an adverse effect of heparin therapy is thrombocytopenia. However, this did not occur as platelet count actually rose from mean pre-treatment level of 237 x 10^9/L to 276.4 x 10^9/L in the 7th post-operative day.

The changes observed in the total white cell count between the pre-operative values and the values obtained on the 1st, 5th and 7th post-operative days, are in keeping with similar changes observed in patients undergoing intermediate and major surgical trauma [13, 14,15]. This change may be attributable to mobilisation of neutrophils from the marginating pool in response to stress of surgery [7] as well as the activation and release of cytokines as a result of surgical trauma [8]. Further increase at the 5th post operative day may be due to wound infection[16].

Salzman [6] wrote on the properties of an ideal prophylactic antithrombotic agent:

"Efficacy in the clinical setting in which it will be employed is foremost with safety (specifically freedom from haemorrhagic side effects) not far behind. The agent should be convenient to administer ... and free of the need for control by laboratory tests ...."

In this trial, efficacy was assessed by a measure of the ability of Enoxaparin to cause hypocoagulable state in terms of prolongation of activated factor X clotting time. This prolongation was translated into heparin units/ml of plasma. For a therapeutic effect, heparin concentration should vary between 0.2 and 0.6u/ml but for prophylactic effect, it should vary between 0.05 and 0.2u/ml [10]. Enoxaparin is maximally effective at two hours post dosing as the clotting time obtained is approximately equivalent to 0.22u/ml of heparin. The efficacy remains within prophylactic range at 24 hours, as the mean heparin concentration at that time was 0.125u/ml and there was no time when the level fell below the prohylactic range. Daily dosing of the drug is therefore adequate for prophylaxis.

Only 2% of the 49 subjects developed features suggestive of deep vein thrombosis. This study is non-comparative but the observed 2% thrombotic episodes compare favourably well with findings in other controlled studies. In a report [17] in 1971, Kakkar et.al found thrombotic phenomenon in 17 (42%) of 39 patients who did not receive and 3 (8%) of 29 who received low-dose heparin prophylaxis. In an international trial [18] in 1975, 164 (25%) of 667 untreated patients and 48 (8%) of 625 others who received low-dose heparin prophylaxis developed thrombosis. In these studies, the end point for detection of thrombosis was investigative (leg scans) and not clinical. This may explain the higher prevalence of 8% of thrombosis as compared with 2% obtained in this trial. However, in a study where low molecular weight heparin was compared with low-dose unfractionated heparin, the group on low molecular weight heparin had lower frequency, 2.5% (5/196), of thrombosis than the group on low-dose heparin with 7.5% (15/199) [19].

The cause of excessive sweating in one patient could not be ascertained. This sweating occurred on days 6 and 7 of therapy soon after administration of trial drug and resolved within 30 minutes on each occasion. This patient may have had hypersensitivity to heparin.

Haematoma of approximately 2cm in diameter was observed at the site of injection in one patient. As there is no apparent alternative cause for this observation, it is believed that this may be a substantive side effect of Enoxaparin, which we now document as occurring in 2% of patients.

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Received: 26 August, 2002
September: 16 September 2004