Graefe's Arch Clin Exp Ophthalmol
(2005) 243: 701–705

LABORATORY INVESTIGATION

DOI 10.1007/s00417-004-0912-0

R. Gary Lane
J. Michael Jumper
Ma'an A. Nasir
Mathew W. MacCumber
Brooks W. McCuen II

A prospective, open-label, dose-escalating study of low molecular weight heparin during repeat vitrectomy for PVR and severe diabetic retinopathy

Received: 7 January 2004
Accepted: 17 February 2004
Published online: 3 March 2005

The authors have no proprietary interest in the material presented and no proprietary interest in the drugs or equipment used in this study. The views expressed in this article are those of the authors and do not reflect the official policy of the Department of Defense or other departments of the United States government. This paper was presented in part at the Association for Research in Vision and Ophthalmology Annual Meeting, Fort Lauderdale, Florida, May 2000.

R. G. Lane · J. M. Jumper
Department of Ophthalmology,
Wilford Hall USAF Medical Center,
San Antonio, TX, USA

M. A. Nasir
California Retina Consultants,
Santa Barbara, CA, USA

M. W. MacCumber
Department of Ophthalmology,
Rush-Presbyterian St. Luke's Hospital,
Chicago, IL, USA

B. W. McCuen II
Duke University Eye Center,
Duke University,
Durham, NC, USA

J. M. Jumper (✉)
West Coast Retina Medical Group,
105 Berry St, Suite 130,
San Francisco, CA, 44107, USA
e-mail: JMJumper@WestCoastRetina.com
Tel.: +415-972-4600
Fax: +415-975-0999

Abstract *Background:* To determine the maximum tolerated dose (MTD) of enoxaparin, a low molecular weight heparin (LMWH) was used during repeat vitrectomy for rhegmatogenous retinal detachment with proliferative vitreoretinopathy (PVR) and severe diabetic retinopathy. *Methods:* From 25 patients, 29 eyes undergoing repeat vitrectomy for PVR (CP3 or greater) or severe diabetic retinopathy were included in the study. Patients had previously undergone an average of 2.1 previous vitrectomies (range 1–5). Enoxaparin was added to the infusion fluid in an escalating dose from 0.1 IU/ml to 6.0 IU/ml as tolerated. Intraoperative bleeding, postoperative fibrin, hyphema and vitreous hemorrhage were graded in an unmasked fashion using previously described grading scales. *Results:* All patients completed the study, and the study was able to achieve the 6.0 IU/ml maximum dose on the dose escalation schedule. No patient experienced dose-limiting toxicity. Analysis showed no increase in intraoperative bleeding complications between low dose (≤1.0 IU/ml) and high dose (>1.0 IU/ml) enoxaparin (Mann-Whitney Test, P=0.029). *Conclusions:* Enoxaparin dose escalation did not result in a dose-dependent increase in acute side effects. The establishment of a well-tolerated dose of enoxaparin during repeat vitrectomy for PVR and severe diabetic retinopathy (6.0 IU/ml) provides a foundation for future studies.

Introduction

Proliferative vitreoretinopathy (PVR) is the leading cause of failure in retinal detachment surgery. Low molecular weight heparin (LMWH), in combination with the antiproliferative agent, 5-fluorouricil, has recently been established as an effective adjunct for prevention of PVR in high-risk patients undergoing primary vitrectomy [1]. The role of LMWH in repeat vitrectomy is unknown. Previous studies have indicated an increased risk of intraoperative bleeding using higher dose heparin.

Formation of intraocular fibrin occurs following approximately one-third of vitrectomies and may jeopardize surgical success by several different mechanisms including pupillary block glaucoma, inferior peripheral iridectomy closure and by providing a scaffold for further proliferation [5, 9]. Currently, there is no effective means to prevent post-vitrectomy fibrin formation. Tissue plasminogen activator has some benefit; however, it can cause intraocular hemorrhage and must be injected into the eye after fibrin has already been deposited [6]. Johnson et al. found heparin to be effective in decreasing post-vitrectomy fibrin formation in a rabbit model [7]. Heparin given within the ocular irrigating solution (4 IU/cc) showed an effect without prolongation of the ocular bleeding time [7]. In a small prospective, randomized clinical trial, Johnson and Blankenship demonstrated that heparin in the ocular irrigating solution during vitrectomy in humans (5 to 10 IU/cc) caused a statistically significant decrease in postoperative fibrin formation with a mild increase in intraocular hemorrhage [8].

Enoxaparin is a low molecular weight heparin composed of depolymerized native heparin molecules with a molecular weight of 4,500 Da, compared to 15,000 Da for native heparin. It was approved in the United States in 1993 for prophylaxis of deep venous thrombosis and is the most studied drug in its class, with a large body of literature describing its safety. Enoxaparin has properties that may be more beneficial than native heparin in ocular surgery. There is an equal antithrombotic effect and reduced hemorrhagic activity when compared to unfractionated heparin [2]. It does not interact with platelet function and does not modify bleeding time. Iverson et al. demonstrated that a LMWH (Fragmin, KabiVitrum AB, Stockholm) given at 5 IU/cc in the ocular irrigating solution markedly inhibited post-vitrectomy fibrin in a rabbit model without increasing intraocular hemorrhage [4]. Although no dose escalation study of intravitreal enoxaparin has ever been performed, a number of previous studies have determined the safe and effective intravenous dose of enoxaparin [3]. These studies have generally found an antithrombotic (anti-fibrinogenic) dose of enoxaparin at 0.1 to 0.2 IU/cc.

Hemorrhagic complications were low at this dose. Because of the theoretical advantages of low molecular weight heparins, a pilot study was undertaken to explore the effect of enoxaparin used in ocular irrigating solution during vitrectomy. As enoxaparin has been previously administered by intravenous infusion in humans [3], and both heparin and low dose enoxaparin have been administered in the ocular irrigating solution in humans [1, 8], we were comfortable in performing dose escalation studies of enoxaparin in the ocular irrigating solution during vitrectomy. Biocompatibility studies were completed by the Pharmacy Department at our institution prior to beginning the study.

Materials and methods

Twenty-nine eyes from 25 subjects requiring repeat vitrectomy were selected for this prospective, open-label dose-escalation study with the objective of determining the maximum tolerated dose (MTD) of intravitreal enoxaparin. Sample size was calculated for a type-I error of 0.05% and a power of 95% of detecting one standard deviation of increase in bleeding severity.

Inclusion criteria were the presence of severe PVR grade CP3 or worse, or severe PDR with tractional retinal detachment and/or anterior hyaloid fibrovascular proliferation (AHFVP). Subjects were included in this phase I study if they were felt to be at high risk of postoperative fibrin and PVR, based on the number of previous vitrectomies, preexisting PVR, diabetes or trauma. Reproductive age females were chosen only if they had a negative preoperative pregnancy test. Minors younger than 18 were not selected. Patients must not have used aspirin or other NSAIDs, other antiplatelet drugs or coumadin within 1 week prior to surgery. All study participants gave informed consent, and this study was approved by our Institutional Review Panel.

Preoperatively, a complete medical history was obtained, including documentation of prior bleeding tendency. A complete physical examination with CBC and platelet counts was performed. The patient population reflected the tertiary referral center where they were enrolled. These patients are not typical of a community-based practice. All of our patients had undergone previous vitrectomy. The average number of previous vitrectomies before enrolling into the study was 2.1, with a range of 1 to 5 prior surgeries. PVR was present in 26 eyes, the remaining 3 eyes had AHFVP. Four eyes had a combination of proliferative diabetic retinopathy (PDR) and PVR (Table 1). Rhegmatogenous retinal detachment was the most common indication for vitrectomy. Other diagnoses included tractional retinal detachment from diabe-

Table 1 Patient characteristics

Dose (IU/cc)	0.1	0.3	1.0	3.0	6.0
Number	3	4	3	7	12
Average age	49	47	51	54	54
Diagnosis:					
Rhegmatogenous retinal detachment with PVR	1	4	2	6	8
Proliferative diabetic retinopathy with PVR	2	0	1	1	4

tes, juvenile rheumatoid arthritis with TRD and PVR and traumatic open globes.

The study purpose was to gain experience with LMWH in a new patient population. The study design was a dose escalation schedule with a pre-determined dose-limiting toxicity as the endpoint. The rationale for this design was to rule out potentially serious complications early in the study (at low doses) and then proceed on to more therapeutically useful doses. Enoxaparin sodium (Lovenox, Aventis Pharmaceuticals, Strasbourg, France) was administered to patients within the standard ocular irrigating solution during vitrectomy as previously performed in an investigation with heparin [8]. We used an initial dose of 0.001 mg/cc or approximately 0.1 IU/cc of irrigating solution. Dose-limiting toxicity was defined as uncontrolled bleeding that prevented completion of the case or any severe systemic adverse effects. If no dose-limiting toxicity was identified in the first three patients, the dose was increased in controlled manner to 0.3 IU/cc in the next three patients and subsequently to 1.0 IU/cc, 3.0 IU/cc and 6.0 IU/cc to complete a clinical response curve. We chose our study dose range based on the well-studied therapeutic intravenous dosage [3] and the highest nontoxic dose of LMWH used in animal studies [4]. Additional surgeries were performed at the 3.0 IU/cc and 6.0 IU/cc dose levels in order to gain additional experience with the drug. As the human eye contains about 4 cc of fluid in the vitreous cavity, at the highest dose (6.0 IU/cc), a maximum of about 24 IU remained in the eye at the conclusion of the procedure. The eye was continuously irrigated with enoxaparin during surgery with a total volume of 500 cc. We used Ringer's lactate plus added 1:1,000,000 epinephrine as our standard irrigating solution, with added 1:20 50% dextrose solution for diabetics.

Average intraoperative manipulations served as an indicator for case length and complexity. One manipulation is given for a vitrectomy, membrane peel, lensectomy, peripheral iridotomy, air-fluid gas exchange, silicone oil, use of perfluorocarbon liquid, retinotomy or endolaser. A 360° retinotomy counted as two manipulations. Intraoperative bleeding was monitored on a semi-quantitative scale as described previously [8] as follows.

Operative bleeding grade

The scale for the operative bleeding grade was: grade 0, bleeding that ceased without intervention and less than 1 DA in extent; grade 1, bleeding that required elevation of the infusion bottle or gradually accumulated to greater than 1 DA in extent; grade 2, bleeding that required elevation of the infusion bottle and one application of diathermy; grade 3, bleeding that required multiple elevations of the infusion bottle and diathermy to several different sites; grade 4, uncontrolled bleeding that did not prevent completion of the procedure; grade 5, uncontrolled bleeding preventing completion of the procedure.

Intraoperative fibrin grade

The scale fo the intraoperative fibrin grade was: grade 0, no fibrin deposition from bleeding upon the retina; grade 1, lightly adherent fibrin deposition from bleeding that was easily aspirated; grade 2, moderately adherent fibrin deposition from bleeding that required aspiration and peeling; grade 3, markedly adherent and/or repeated fibrin deposition from bleeding that required meticulous peeling.

Postoperative day 1 fibrin grade

The scale for the postoperative day 1 fibrin grade was grade 0, no fibrin; grade 1, short fibrin strands adherent to the iris or lens; grade 2, fibrin filling less than 50% of the pupil or peripheral iridectomy or forming a transvitreal strand; grade 3, fibrin filling the pupil and/or peripheral iridectomy or forming a few transvitreal strands; grade 4, severe fibrin filling at least 25% of the anterior chamber or forming extensive transvitreal strands.

Postoperative day 1 hyphema

Postoperative day 1 hyphema was expressed as a percentage.

Postoperative day 1 vitreous hemorrhage grade

The scale for the postoperative day 1 vitreous hemorrhage grade was grade 0, no hemorrhage; grade 1, hemorrhage partially obscuring the retina; grade 2, the view of retinal details hazy but visible; grade 3, no view of the retina.

Safety assessment rules

We used the following rules to guide the assessment of safety and to permit increases in dose concentration: (1) If any excessive intraoperative hemorrhage occurred, the enoxaparin-containing irrigating solution would be discontinued. (2) If no toxicity develops within a dose level, proceed to the next level. (3) If one patient experiences mild to moderate

Table 2 Comparison of dose groups

	Low dose	High dose
Average age	51	54
Diabetic	6/10	8/19
Previous vitrectomies	2.2	2.1
Average intraoperative manipulations	7.1	6.4

Table 3 Intra-operative bleeding complications

	Low dose	High dose
Intra-operative bleeding ≥ grade 2	3/10 (30%)	4/19 (21%)
Any intra-operative bleeding	9/10 (90%)	8/19 (42%)

Table 4 Postoperative day 1. Bleeding complications

	Low dose	High dose
Postoperative hyphema >10%	2/10 (20%)	3/19 (16%)
Any vitreous hemorrhage	4/10 (40%)	5/19 (26%)

toxicity, repeat at the same dose level. (4) If two or more patients experience moderate toxicity, stop the dose escalation and revert to the previous dose level. (5) If any patient at any dose level has dose-limiting toxicity, stop escalation. (6) Toxicity definitions are: mild, includes bleeding that is readily controllable by conventional treatments, including elevation of intraocular pressure; moderate, includes bleeding that is more severe than expected, more prolonged than expected, any systemic toxicity (e.g., bruising), rash and non-life threatening allergic reactions; severe, includes, but is not limited to, unexpected bleeding or severe bleeding (systemic or ocular), unexpected pain, new lesions possibly related to drug administration, unexpected changes in visual acuity or function, nerve damage or precipitation in ocular fluids or severe allergic reactions. (7) Dose-limiting toxicity was defined specifically as grade 5 intraoperative bleeding (uncontrolled intraocular bleeding that prevented completion of the procedure) or any severe systemic reaction.

Postoperatively, detailed examinations were performed at 1 day, 1 week and 3 weeks post-vitrectomy. The extent of hemorrhage, the extent of fibrin formation, corneal clarity and closure of an inferior iridectomy were assessed on a semi-quantitative scale. Postoperatively, medical history was obtained to screen for systemic bleeding episodes.

Results

Twenty-nine of 29 eyes completed the study, and the study was able to achieve the 6.0 IU/ml maximum dose on the dose-escalation schedule. No patient experienced dose-limiting toxicity. Analysis showed no increase in intraoperative bleeding complications between low dose (≤1.0 IU/ml) and high dose (>1.0 IU/ml) enoxaparin. Results were grouped into two categories for analysis. The low dose group included the first ten patients and used doses from 0.1 to 1.0 units per ml. Doses of 3.0 to 6.0 units per ml were categorized as high dose and included the last 19 patients.

There were no statistically significant differences between the two groups in terms of age, diagnosis, number of intraoperative manipulations or number of prior vitrectomies (Table 2). By chance, a higher percentage of diabetics (6/10) were found to be in the low-dose group than in the high-dose group (8/19). However, the percentage of surgical cases with PDR was identical between the two groups (3/10 in low dose vs. 6/19 in the high dose).

The incidence of intraoperative bleeding is shown in Table 3. The results were analyzed using the Mann-Whitney test, a standard statistical method for non-parametric data. The graded results for intraoperative bleeding for all 29 eyes were rank ordered based on severity, and the average ranking for the high and low dose groups were compared. Our results showed that the high dose group did not have increased intraoperative bleeding severity compared to the low dose group (P=0.029). The graphic results of the mean rank test used to analyze our data are shown in Fig. 1. Postoperative day 1 bleeding complications were analyzed by the same method (Table 4). There were no significant differences in bleeding complications between the high and low dose groups. Postoperative fibrin formation also showed no significant difference between dose groups (Table 5).

Of 29 patients enrolled, only one patient in the 0.3 IU/cc dose group had more intraoperative bleeding than expected (termed "moderate" under the protocol). This patient experienced a grade 4 intraoperative hemorrhage, which did not prevent completion of the case.

In accordance with study protocol, an additional case at this dose level was completed without incident prior to dose escalation. We do not believe this was due to the drug since it continued after the enoxaparin was terminated and was well washed from the eye. There was no sign of ocular toxicity or systemic effects of the drug in any eye.

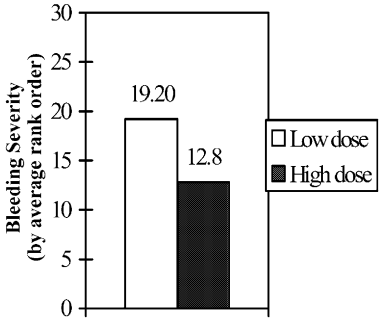

Fig. 1 Severity of intraoperative bleeding. A comparison of intraoperative bleeding severity between the low dose group (shown in *white*) and the high dose group (in *black*) using the Kruskal-Wallis mean rank test. A higher score means worse intraoperative bleeding complications

Table 5 Postoperative day 1. Fibrin complications

	Low dose	High dose
Postoperative fibrin ≥ grade 2	2/10 (20%)	3/19 (16%)
Peripheral iridotomy closure	2/6 (33%)	2/14 (14%)

Discussion

PVR and postoperative fibrin remain significant obstacles in the treatment of retinal detachment. Previous attempts to prevent or treat fibrin with heparins have been successful, but show increased bleeding [8]. More recently, a study of PVR prevention in high-risk patients with primary rhegmatogenous retinal detachment showed a beneficial effect of using enoxaparin in combination with the antimetabolite, 5-fluorouracil [1]. The investigators used a single dose of enoxaparin (5.0 IU/ml) and noted no adverse effects. The authors do not state the rationale for the enoxaparin dose used. Their study population differed from ours in that PVR was not present and, therefore, manipulations of the retina such as membrane peeling and retinotomy were less often performed. To date, no dose escalation study for intravitreal enoxaparin has been performed. The purpose of a phase I dose-escalating study is to determine how large a dose (the maximally tolerated dose, MTD) can be given before toxicity is experienced by patients. This study design is most often employed in the cancer literature. In these studies, a small number of patients, typically three, are entered sequentially at a particular dose. If no specified level of toxicity is observed in any of the three patients, three more patients are treated at the next predetermined dose. We are able to report no serious ocular or systemic side effects at any dose of enoxaparin.

Enoxaparin does not appear to increase bleeding at increased doses. This is in contrast to previous studies with native heparin, which showed greater bleeding at higher doses [8]. It is possible that this or any phase I study could miss toxicity that is minor and uniform across the dosage range. We feel that this is less likely for a drug with a well-understood molecular activity and with which there is considerable experience in animal eyes (LMWH) and in human eyes (other heparins) [4, 7, 8].

Our results would indicate that intraocular enoxaparin may be used safely during vitrectomy at doses up to 6.0 units per ml in a standard ocular irrigating solution without increasing bleeding complications in comparison to low dose enoxaparin. We feel that if LMWH has a therapeutic effect, it will be in this dosage range. Accordingly, we did not study higher doses to find a toxic effect. Although there was less fibrin than one might expect from this population, we are not able to assess the efficacy of infused low molecular weight heparin without a randomized, controlled phase II study. This study was designed to gain experience with LMWH in complex patients as a prelude to a randomized controlled trial. A broad spectrum of conditions and surgical manipulations were included in each dosage group. This variety of patients is advantageous in a dose-escalation study to look for any possible situations where toxicity might occur. Because of the study design, however, care should be taken not to extrapolate results beyond the stated objective of determining a maximum tolerated dose. Claiming efficacy is very different from demonstrating lack of toxicity. As this was an uncontrolled phase I study using unmasked grading, we are unable to draw conclusions about efficacy. Future studies should include control groups and ensure uniformity of subjects between various study groups. The statistical power of the study was sufficient to detect a large, clinically significant dose-limiting toxicity, and none was encountered.

The lack of intraoperative bleeding complications at higher doses of LMWH is a welcome finding. During vitrectomy, high-risk patients can rapidly form a thick fibrin clot that is very difficult to remove, which can cause additional bleeding and prolongs operating time, all of which increases fibrin and PVR. Heparin could have the potential to make high-risk surgery easier by rapidly eliminating thrombin before a dense clot can form. This study provides valuable experience with LMWH in vitreoretinal surgery. Based on these encouraging results, we feel that LMWH shows promise in vitreoretinal surgery and should be studied further.

References

1. Asaria RH, Kon CH, Bunce C, Charteris DG et al (2001) Adjuvant 5-fluorouracil and heparin prevents proliferative vitreoretinopathy. Ophthalmol 108:1179–1183
2. Breddin HK (1989) Low molecular weight heparins and bleeding. Semin Thromb Hemost 15:401–404
3. Huet Y, Gouault-Heilmann M (1986) Low molecular weight fraction PK 10169: A new therapeutic means for anticoagulant therapy? Haemostasis 16:165–172
4. Iverson DA, Katsura H, Hartzer MK, Blumenkranz MS (1991) Inhibition of intraocular fibrin formation following infusion of low-molecular-weight heparin during vitrectomy. Arch Ophthalmol 109:405–409
5. Jaffe GJ, Schwartz D, Han DP, et al (1990) Risk factors for postvitrectomy fibrin formation. Am J Ophthalmol 109:661–667
6. Jaffe GJ, Abrams GW, Williams GA, Han DP. (1990) Tissue plasminogen activator for postvitrectomy fibrin formation. Ophthalmology 97:184–189
7. Johnson RN, Balyeat E, Stern WH (1987) Heparin prophylaxis for intraocular fibrin. Ophthalmology 94:597–601
8. Johnson RN, Blankenship G (1988) A prospective, randomized, clinical trial of heparin therapy for postoperative intraocular fibrin. Ophthalmology 95:312–317
9. Madreperla SA, McCuen BW (1995) Inferior peripheral iridectomy in patients receiving silicone oil: rates of postoperative closure and effect on oil position. Retina 15:87–90