External/Visceral Examination of Fetuses. Data expressed as %Incidence/Number of Litters.

Parameters	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
Fetuses/Litters	73/11	87/12	82/11	49/7	108/13
Hemorrhage on gall bladder	2.7%/1	3.4%/2	6.1%/4	10.2%/4	1.9%/2
Small fetus (<32.0 g)	21.9%/5	50.6%/8	40.2%/8	57.1%/5	30.6%/8

Skeletal Examination of Fetuses. Data expressed as %Incidence/Number of Litters.

Parameters	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
Head					
Fetuses/Litters	52/11	60/12	57/11	34/7	76/13
Large anterior fontanelle	0	3.3%/2	1.8%/1	5.9%/2	0
Posterior fontanelle enlarged	5.8%/2	13.3%/5	7.0%/2	17.6%/2	7.9%/4
Small unossified area in parietal bone	0	0	3.5%/2	8.8%/1	0
Small discrete unossified area in frontal bone	0	0	0	2.9%/1	0
Acephaly	0	0	0	2.9%/1	0
Hyoid bone incompletely ossified or unossified	9.6%/4	36.7%/8	38.6%/8	35.3%/5	30.3%/9
Sternebrae and Ribs					
Fetuses/Litters	73/11	87/12	82/11	49/7	108/13
Incomplete ossification of 1 sternebrae	17.8%/7	50.6%/12	25.6%/8	28.6%/6	29.6%/12
Incomplete ossification of 2 sternebrae	2.7%/2	4.6%/3	4.9%/3	16.3%/2	3.7%/3
Incomplete ossification of 3 sternebrae	0	1.1%/1	0	2.0%/1	0
Ribs 12/12	41.1%/9	65.5%/12	64.6%/10	71.4%/7	45.4%/12
Vertebrae, Limbs, and Girdles					
Fetuses/Litters	73/11	87/12	82/11	49/7	108/13
Incomplete ossification of 2rd thoracic vertebral centrum	0	0	0	2.0%/1	0
Incomplete ossification of caudal vertebrae, less than 16 ossified	0	0	1.2%/1	6.1%/1	0
25 pre-sacral vertebrae	0	0	0	2%/1	0
One or both centrales completely ossified or unossified	1.4%/1	6.9%/3	6.1%/2	14.3%/2	0
Incomplete ossification of metacarpals/ phalanges	11.0%/4	28.7%/8	22.0%/8	32.7%/4	19.4%/8

Anomalous forelimb flexure	0	0	1.2%/1	2.0%/1	0.9%/1
Pubic bones incompletely ossified or unossified	0	5.7%/3	6.1%/2	14.3%/2	0
Double association pelvis, ilial bones associated with both sacral vertebrae	0	1.1%/1	1.2%/1	4.1%/2	0.9%/1

Fetal Head Examination. Data expressed as %Incidence/Number of Litters.

Parameters	**0 mg/kg/day**	**4 mg/kg/day**	**10 mg/kg/day**	**25 mg/kg/day**	**Heparin**
Fetuses/Litters	21/11	27/12	25/10	15/7	32/13
Lower incisors only erupted	9.5%/2	11.1%/3	16.0%/3	13.3%/2	28.1%/8
Blood hemorrhage in nasal sinuses/ nasopharynx	0	11.1%/2	8.0%/2	6.7%/1	0
Unilateral slightly folded retina	4.8%/1	0	4.0%/1	13.3%/1	3.1%/1
Bilateral slightly folded retina	0	0	0	0	3.1%/1
Blood in cochlea(s)	9.5%/1	18.5%/4	20.0%/3	20.0%/3	21.9%/5

In a subcutaneous Segment II subcutaneous teratology study, pregnant female rats received tinzaparin at doses of 0, 4, 10, or 25 mg/kg/day from days 6 to 19 of gestation. Heparin at a subcutaneous dose of 12.5 mg/kg/day was administered to a group of pregnant female rabbits as a comparator. Tinzaparin at subcutaneous doses ≤25 mg/kg/day was not teratogenic in rabbits. Mortality occurred for 1 dam in each of the tinzaparin treatment groups and 1 dam in the heparin comparator group. The incidence of spontaneous abortion and premature delivery was increased for dams receiving tinzaparin at 25 mg/kg/day, which was not observed in the dose range finding study. Further, body weight gain was suppressed in all tinzaparin groups as well as the control group during and after the treatment period, which resulted in the sponsor conducting a second teratology study in rabbits, which is described below. External examination found that the incidences of small fetuses (<32.0 g) were increased for tinzaparin treatment groups. Skeletal examination found increased incidences of variations for tinzaparin treatment groups, which consisted of incompletely ossified/unossified for bones in the head, sternebrae and ribs, and vertebrae, limbs, and girdles. Examination of fetal heads found that tinzaparin at all dose levels increased the incidence of blood in cochlea(s); although, there was not a dose response relationship.

Segment II: (Second) Teratology Study in the Rabbit (LSR Report # 89/NLP083/178).

Conducting Lab: [redacted]

Dates of Conduct: Initiated 10/18/88, completed 11/24/88.

GLP Statement: In compliance with the FDA's GLPs.

Chemical: LHN-1, lot #s 830030, -31, and -32; and corresponding batch #s 704Y; and Heparin lot # 829931.

Animals: Pregnant Rabbits, New Zealand white rabbits; 15/dose group.

Doses: 0 (control vehicle), 2. 8, and 25 mg/kg/day LHN-1, and Heparin at 12.5 mg/kg/d, given subcutaneously in 0.5 mL/kg volume, at different sites, once daily on days 6 through 19 of gestation. These doses were selected from the Preliminary Teratology Study (87-NLP028/932) using 4 pregnant rabbits/dose given subcutaneous LHN-1 at 10 and 25 mg/kg/d on days 6-19 of gestation. There were no fetotoxic or teratogenic effects in the rabbits.

Methods: The dams were given the vehicle or the test agent once daily on days 6 through 19 of gestation. The dams were observed for morbidity and mortality (and standard observations of general appearance, body weights, food intake and abortions) daily throughout the pregnancy until day 29 when they were killed and their uteri examined for implantation sites. It should be noted that blood samples were collected 24-hr post-dosing on days 6, 19 and 28 for determining packed cell volume, Hb concentration, and erythrocyte counts. The following parameters were recorded: pregnancy rates, number of animals aborted, implantation sites, number and distribution of live/dead fetuses, intrauterine live/dead embryos, corpora lutea and ovarian weight. Fetal examinations consisted of fetal/placental weights and sex ratio, abnormal litter ratio, malformation rate, and ossification variance. One-third of the fetuses from each litter were examined for visceral and 1/3 for skeletal anomalies (by Bouin's and Alizarin's techniques). The remainder 1/3 fetuses' heads were examined for abnormalities by serial sectioning. Detailed histopathology of the rabbits that died during the study was also performed. The mean fetal weight, placental weight, post-implantation loss, and sex ratios were analyzed on the fetus basis.

Results: One control and one high dose dam were killed due to reduced food intake, weight loss and necropsy showed hemorrhages at injection sites, and clotted blood in the uterus and vagina of the high dose dam.

Body Weight Gains in the low, mid and high dose group pregnant rabbits were reduced during the first 12-18 days of gestation; however, the rabbits gained body weight normally during the latter part of gestation. In the Heparin group, body weight gain remained significantly reduced throughout gestation. Food intake paralleled with body weight gain.

Hematology: There was a significant reduction in the Hb concentration, the packed cell volume and erythrocyte counts in the 8 and 25 mg/kg/d dose LHN-1 and Heparin treated dams on day 20 and 28 of gestation (with slight recovery on day 28). The packed cell volume (%), Hb (g%), and erythrocyte counts (mil/cmm) on day 28 were: 35, 11.2 and 5.01 in the high dose group, compared to 39, 12.4, and 5.71 in the controls. The low dose did not show such hematotoxic effects.

Findings at Autopsy: Subcutaneous hemorrhages at injection sites were commonly seen in all the treated rabbits. One dam each in the 8 and 25 mg/kg/d LHN-1 and Heparin group aborted; and one dam in the low dose group delivered prematurely.

The fetal weights in the mid and high dose LHN-1 and Heparin group were reduced slightly, but not significantly. The other parameters such as: implantations, the number of live fetuses born/litter, pre- and post-implantation losses and placental weights in the treatment groups did not differ significantly from the controls.

The incidence of malformations and skeletal variations in the treatment groups was also comparable with controls. Interestingly, the number of fetuses with blood in the cochlea was significantly higher in the treated groups than in the controls. The incidence was: 1/3, 7/14, 7/16, 14/23, and 3/5 fetuses examined in the control, 2, 8, 25 mg/kg/d LHN-1 and the Heparin group, respectively. It is not clear whether this effect was related to LHN-1 treatment. The sponsor stated that this could be due to the way fetal heads were sectioned for processing.

In summary, the treatment of rabbits with LHN-1 at doses of 2, 8, and 25 mg/kg/d and Heparin at 12.5 mg/kg/d produced some maternal toxicity (reduced maternal weight gain initially), and hematotoxicity (reduced Hb, packed cell volume, and erythrocyte counts) at 8 and 25 mg/kg/d doses but not at 2 mg/kg/d. Fetal toxicity was evidenced by decreased fetal weights at 25 mg/kg/d of LHN-1 and hemorrhaging cochlea at all doses. However, LHN-1 did not produce any clear signs of malformations in the rabbits at doses of up to 25 mg/kg/d and neither did Heparin at 12.5 mg/kg/d. But it should be noted that the test agent used in this study was from lot #s 830030, 830031, and 830032 and the corresponding batch #s 704Y, and the lot # of Heparin tested was # 829931 that were not tested before in any study.

Addendum:

Testing Laboratory: [redacted]

Drug Batch: Tinzaparin bulk drug batch F704Y ([redacted] anti-Factor Xa IU/mg) was supplied as a solution that contained sodium metabisulfite. The following lot numbers were used: 830030, 830031, and 830032.

Doses: Doses were equivalent to 0, 200, 600, and 1900 anti-Factor Xa IU/kg/day, respectively.

Dose Selection: In a Segment II subcutaneous dose range finding study, pregnant female rabbits received tinzaparin at 25 mg/kg/day (Batch No. F668A) or at 25 and 50 mg/kg/day (Batch No. F682X) from days 6 to 19 of gestation (LSR Report No.: 89/NLP082/063). Vehicle-control and positive control groups received [redacted] respectively. There were no treatment-related effects on body weight gain. Body weights of fetuses from maternal rabbits treated with tinzaparin at 25 or 50 mg/kg/day (Batch No. F682X) were decreased to 84.8 and 94.5% of the control (40.2 g), respectively; however, body weights of fetuses from maternal rabbits treated with 25 mg/kg/day (Batch No. F668A) were unaffected. The incidences of small fetuses (<32.0 g) for tinzaparin (Batch No. F682X) at 25 or 50 mg/kg/day were increased to 33.3%/4 litters and 24.2%/4 litters, respectively, as compared to 18.4%/ 2 litters for the control group. The incidence of acute inflammatory cells in the myometrium were increased for tinzaparin (Batch No. F682X) at 50 mg/kg/day. Chronic inflammatory cells in the myometrium were observed for all groups.

Disposition of Animals: One female rabbit that received tinzaparin at 25 mg/kg/day was sacrificed in a moribund condition on day 19 of gestation. Necropsy revealed extensive subcutaneous hemorrhages and clotted blood in the genital tract. One control rabbit was sacrificed in a moribund condition on day 27 following a marked weight loss.

Disposition of rabbits treated with tinzaparin or heparin.

Parameter	0 mg/kg/day	2 mg/kg/day	8 mg/kg/day	25 mg/kg/day	Heparin
Total number inseminated	15	15	15	15	15
Mortality	1	0	0	1	0
Not Pregnant	0	0	1	1	0
Abortion/Premature Delivery	0	1	0	1	1
Pregnant at term with viable young	14	14	14	12	14

Body Weight and Food Consumption: There were no apparent treatment-related effects on body weight gain. Body weights for female controls on days 6 and 20 of gestation were 4.39 and 4.67 g, respectively, yielding a 6.4% increase of body weight from day 6. Body weights for tinzaparin-treated rabbits at 2, 8, and 25 mg/kg/day on day 20 of gestation were increased by 5.6, 5.2, and 4.9%, respectively, of body weights on day 6. Food consumption for tinzaparin-treated rabbits at 25 mg/kg/day from days 6-12 and days 13-19 was decreased to 89.5 and 81.9% of control values (209 and 182 g/rabbit/day), respectively.

Litter Data for Female Rabbits Killed on Day 29 of Gestation: Body weights of fetuses obtained from maternal rabbits treated with tinzaparin at 25 mg/kg/day were decreased to 91.5% of the control (41.2 g).

Parameter	0 mg/kg/day	2 mg/kg/day	8 mg/kg/day	25 mg/kg/day	Heparin
#Pregnant Animals	14	15	14	13	15
% Abortion and Premature Delivery	0	6.7	0	7.7	6.7
Corpora lutea/dam	13.2	13.0	11.9	12.2	12.4
Implantation sites/dam	11.5	11.4	11.1	11.4	10.1
Viable fetuses/dam	9.0	10.1	9.4	10.3	8.9
Resorptions					
-early	0.9	0.5	1.4	0.8	0.3
-late	1.6	0.7	0.4	0.3	0.9
-total	2.5	1.2	1.7	1.2	1.1
Pre-implantation loss, %	13.0	12.6	6.6	6.2	18.5
Post-implantation loss, %	21.7	10.7	15.4	10.2	11.3
Fetal body weight, g	41.2	39.8	40.3	37.7	37.9
Placental weight, g	5.6	5.3	5.6	5.3	6.3

External and Skeletal Examinations of Fetuses and Visceral Examinations of Fetal Heads: Examination of fetal heads found that the incidence of blood in cochlea(s) of fetuses from maternal rabbits treated with tinzaparin at 2, 8, and 25 mg/kg/day was increased as compared to the control. The sponsor attributed this finding to decapitation process used; however, under these circumstances, it would be expected that the incidence of blood in cochlea(s) would be increased for all groups. There were no treatment-related findings with external, visceral, and skeletal examinations of fetuses.

External/Visceral Examination of Fetuses. Data expressed as % Incidence/Number of Litters.

Parameter	0 mg/kg/day	2 mg/kg/day	8 mg/kg/day	25 mg/kg/day	Heparin
Fetuses/Litters	126/14	142/14	132/14	123/12	125/14
Possible domed head	0	0	0	0.8%/1	0
Opaque areas in centers of both eyes	0	0	0.8%/1	0.8%/1	0
Heart appears large, excess pericardial fluid	0	0	0	0.8%/1	0
Clear/red serous fluid/clotted blood in abdomen	0	0.7%/1	0	0.8%/1	0.8%/1
Small fetus (<32.0 g)	17.5%/7	21.8%/7	25.8%/7	22.8%/7	19.2%/11
Pale area on placenta	0	0	0	0.8%/1	4.8%/1

Skeletal Examination of Fetuses. Data expressed as % Incidence/Number of Litters.

Parameter	0 mg/kg/day	2 mg/kg/day	8 mg/kg/day	25 mg/kg/day	Heparin
Head					
Fetuses/Litters	84/14	98/14	89/14	86/12	83/14
Medium anterior fontanelle	57.1%/13	53.1%/13	60.7%/14	74.4%/12	75.9%/14
Large anterior fontanelle	0	1.0%/1	0	3.5%/3	2.4%/2
Incomplete ossification of supra-occipital bone	0	1.0%/1	0	3.5%/3	1.2%/1
Interparietal bone fissured	0	0	0	1.2%/1	1.2%/1
Additional suture in parietal bone	0	1.0%/1	0	1.2%/1	0
Small discrete unossified area in frontal bone	0	0	0	1.2%/1	0
Additional suture in nasal bone	0	0	0	1.2%/1	0
Irregular ossification of frontal suture	0	0	1.1%/1	3.5%/3	4.8%/4
Frontal suture enlarged at fronto-nasal junction	0	0	0	1.2%/1	1.2%/1
1st cervical vertebral centrum incompletely ossified	0	0	0	1.2%/1	0
Sternebrae and Ribs					
Fetuses/Litters	126/14	142/14	132/14	123/12	125/14
Incomplete ossification of 3 sternebrae	0	0	0	1.6%/2	0
Vertebrae, Limbs, and Girdles					
Fetuses/Litters	126/14	142/14	132/14	123/12	125/14
Incompletely ossified or assymetric costal elements of sacral vertebrae	3.2%/3	2.8%/4	4.5%/3	7.3%/5	4.0%/5
One caudal vertebra offset, tail tip kinked	0	0	0	0.8%/1	0
Additional ossification of olecranon processes	0.8%/1	0.7%/1	3.0%/3	2.4%/3	1.6%/2

Centrales incompletely ossified or unossified	0	2.1%/2	0.8%/1	1.6%/2	3.2%/2
Metacarpals and/or phalanges incompletely ossified or unossified	11.9%/8	15.5%/7	18.2%/7	19.5%/10	13.6%/9
Pubic bones unossified	0	0.7%/1	0	1.6%/2	1.6%/2
Assymmetric pelvis, ilial bones associated with different sacral vertebrae	3.2%/3	2.8%/4	3.0%/3	4.9%/3	2.4%/3

Visceral Examination of Fetal Head. Data expressed as % Incidence/Number of Litters.

Parameter	0 mg/kg/day	2 mg/kg/day	8 mg/kg/day	25 mg/kg/day	Heparin
Fetuses/Litters	42/14	48/15	43/14	37/12	42/14
Lower incisors only erupted	11.9%/5	8.3%/4	9.3%/4	27.0%/7	4.8%/2
Unilateral slightly folded retina	11.9%/5	8.3%/4	18.6%/7	18.9%/6	9.5%/3
Blood in cochlea(s)	2.4%/1	18.8%/4	18.6%/5	40.5%/7	7.1%/2

In a subcutaneous Segment II teratology study, pregnant female rabbits received tinzaparin at doses of 0, 2, 8, and 25 mg/kg/day from days 6 to 19 of gestation. One female rabbit that received tinzaparin at 25 mg/kg/day was sacrificed in a moribund condition on day 19 of gestation. Tinzaparin at subcutaneous doses ≤25 mg/kg/day was not teratogenic in rabbits. Body weights of fetuses obtained from maternal rabbits treated with tinzaparin at 25 mg/kg/day were decreased to 91.5% of the control (41.2 g). Examination of fetal heads found that the incidence of blood in cochlea(s) of fetuses from maternal rabbits treated with tinzaparin at 2, 8, and 25 mg/kg/day was increased as compared to the control. The sponsor attributed this finding to decapitation process used; however, under these circumstances, it would be expected that the incidence of blood in cochlea(s) would be increased for all groups. Further, this finding was observed in the first Segment II subcutaneous teratology study in rabbits.

Rats

Segment III: Intravenous Perinatal and Postnatal Development Study in the Rat (LSR Report No.: 92/NLP139/0524).

Testing Laboratory: [redacted]

Date Started: October 2, 1991 (Animals received)

Date Completed: November 17, 1992

GLP Compliance: Statements of compliance with GLP Regulations and the Quality Assurance Unit were included.

Animals: Pregnant female Sprague-Dawley rats (CD strain) were used in this study. At the start of the study, animals were 9-10 weeks of age and had a body weight range of 215-289 g.

Drug Batch: Tinzaparin, Lot No. LMW 9101 (—anti-Factor Xa IU/mg).

Methods: In an intravenous Segment III perinatal and postnatal development study, pregnant female Sprague-Dawley rats received tinzaparin at doses of 0, 5, 20, and 75 mg/kg/day (equivalent to 0, 400, 1700, and 6500 anti-Factor Xa IU/kg/day, respectively) from days 17 to 20 of gestation and days 1 to 25 postpartum. Control animals received the vehicle, [redacted]. There were 24 pregnant female rats/group. The sponsor's dose selection was based upon a preliminary Segment I/III dose range finding study (LSR Report No.: 92/NLP134/0093), which was previously described in the methods section of the Intravenous Segment I fertility and reproductive performance study in rats (LSR Report No.:92/NLP137/0528). In the present study, vehicle or drug solution was administered by the intravenous route at a dose volume of 1 mL/kg. Treatment was suspended immediately prior to and during parturition to minimize the potential risk of excessive bleeding from the uterus. Animals were observed daily throughout the study for clinical signs of toxicity. Any animals found dead or sacrificed in moribund condition were subjected to a complete macroscopic examination of the visceral organs. Body weights of Pregnant F_0 dams were measured on days 0, 6, 10, and 14 of gestation and daily from day 17 of gestation until parturition. Food consumption of pregnant F_0 dams was measured on days 0-2, 3-5, 6-9, 10-13, 14-16, and 17-20 of gestation. F_0 dams were allowed to deliver and rear their offspring. From day 20 of gestation, F_0 dams were observed two to three times per day for onset, progression, and completion of parturition. Individual gestation lengths were calculated. Body weights of F_0 dams were measured daily from days 1 to 26 postpartum. Food consumption of F_0 dams was measured on days 1-3, 4-6, 7-10, 11-13, 14-17, 18-20, and 21-24 postpartum. Methods for monitoring development of F_1 offspring are described in the Methods section of Report No. 92/NLP150/0828.

Results:

1. **Observed Effects for F_0 Dams**: Signs of pallor and swelling or hematoma were observed for several F_0 female rats at 20 and 75 mg/kg/day. There were no observed effects at 5 mg/kg/day.

2. **Mortality of F_0 Dams**: Mortality occurred with doses of 20 and 75 mg/kg/day. At 20 mg/kg/day, three female rats were sacrificed in a moribund condition. At 75 mg/kg/day, 7 female rats were sacrificed in a moribund condition and 1 female rat was found dead. Internal macroscopic examinations of dams at 20 and 75 mg/kg/day, sacrificed in a moribund condition or found dead, revealed evidence of hemorrhage.

Moribund Sacrifices and Deaths at 20 and 75 mg/kg/day.

Dose, mg/kg/day	Animal #	Mode of Death	Day of Death	Outcome for Litter	Internal Findings
20	1050	MS	25 pp	Weaned at day 25	Subcutaneous hematoma, all organs pale
20	1053	MS	4 pp	Terminated	All organs pale, thymus gland hemorrhagic, and hematoma in urinogenital area
20	1063	MS	14 pp	Terminated	Subcutaneous clotted blood around urinogenital area
75	1067	FD	6 pp	Terminated	Abdominal cavity filled with free blood
75	1070	MS	6 pp	Terminated	Hematoma in urinogenital area
75	1074	MS	19 g	Terminated	Pale organs, blood in both uterine horns
75	1079	MS	22 g	Terminated	Sacrificed during parturition; all organs pale
75	1081	MS	18 g	Terminated	Free blood in vagina and cervix. Large amount of red serous fluid in uterine horns. All internal organs pale.
75	1085	MS	24 pp	Weaned at day 24	Hematoma in lower abdominal fat. Cross section revealed clotted blood.
75	1087	MS	6 pp	Terminated	
75	1096	MS	21 pp	Weaned at day 21	Swelling on lower dorsal thorax resulting from hemorrhagic tissue

Abbreviations: MS = Moribund Sacrifice; FD = Found Dead; pp = postpartum, and g = gestation.

3. **Body Weight and Food Consumption for F_0 Dams**: Body weight gains and food consumption for F_0 dams at 75 mg/kg/day from days 17 to 21 of gestation were impaired. Body weight gains and food consumption for F_0 dams from days 1 to 25 postpartum were unaffected by treatment. Body weights of control dams on days 17 and 21 of gestation were 375 and 442 g, respectively. Body weight gains for F_0 dams at 5, 20, and 75 mg/kg/day from days 17 to 20 of gestation were 102.6, 97.4, and 89.1% of the control, respectively. Food consumption for dams at 75 mg/kg/day from days 17 to 20 of gestation was reduced to 93.5% of the control (31 g/animal/day). Body weights of control dams on days 1 and 25 postpartum were 323 and 342 g, respectively. Body weight gains of dams at 5, 20, and 75 mg/kg/day from days 1 to 25 postpartum were 138.25, 144.7, and 105.6% of the control, respectively.

4. **Litter Parameters for F_0 Dams Allowed to Deliver Their Offspring**: The gestation index at 75 mg/kg/day was slightly reduced. There were no treatment-related effects on length of gestation. Necropsy examinations of F_0 dams sacrificed after day 25 postpartum found no treatment-related effects. Necropsy examination of F_0 dams at 20 and 75 mg/kg/day found blue staining and/or bruising on the tail

Gestation Index.

Parameters	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
#Pregnant animals	24	24	24	22*
#Live litters born	24	24	24	21
Gestation Index, %	100	100	100	95

*Excludes two pregnant F_0 dams killed before parturition.

Necropsy Examination of F_0 Female Dams after Day 25 Postpartum.

Findings	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
Swelling and bruising in perianal area	0	0	1	1
Subcutaneous clotted blood in perianal area	0	0	0	1
Blue staining and/or bruising on tail	0	0	2	2
Lumbar lymph nodes congested	0	0	0	1
Thymus enlarged and hemorrhagic	0	0	0	1
Clear fluid cysts and raised white areas on spleen	0	0	0	1

5. **Viability, Growth, and Development of F_1 Pups**: The lactation indexes at doses of 20 and 75 mg/kg/day on day 25 were reduced to 95 and 86%, respectively; however, these effects appeared to be directly linked to maternal toxicity. At 20 mg/kg/day, two litters were litters were terminated, one on day 4 postpartum and the other on day 14 postpartum due to the moribund sacrifice of F_0 dams. At 75 mg/kg/day, three litters were terminated on day 6 postpartum due to death or moribund sacrifice of F_0 dams. Body weight gains for male and female F_1 pups from days 1 to 25 postpartum were reduced to 93.4 and 93.5% of the control, respectively. Body weight gains of F_1 male and female pups, not selected for assessment of reproductive function, from weeks 5 to 9 were unaffected by treatment; although, absolute body weights of F_1 pups at 75 mg/kg/day tended to be slightly lower than that of controls. The male to female pup ratio at 75 mg/kg/day prior to culling on day 4 (1:1.14 to 1:1.16) appeared to be different from the ratio observed for the control (1:1.01). There were no treatment-related effects on implantation sites/dam, the post-implantation survival index, the birth index, the live birth index, or the viability index on day 4 postpartum. There were no treatment-related effects on offspring development (i.e., pinna unfolding, hair growth, testes descent, tooth eruption, eye opening, or vaginal opening). There were no treatment-related effects on auditory and responses (i.e., normal auditory startle response, normal visual placing response, normal pupil closure response). Locomotor activity and performance in the water maze test were unaffected by treatment. Neuromuscular function, as assessed with stationary rods, rotarod treadmill, grid gripping, wire hanging, and mid-air righting reflex, were unaffected by treatment. Necropsy examination of F_1 pups that died prior to the week 9 scheduled termination included findings of all digits cyanosed, cecum enlarged, and unilateral hydronephrosis. For F_1 pups sacrificed at the week 9 scheduled termination, there were findings of bilateral hydronephrosis and pedunculate red body attached to testicular fat.

F_1 Offspring Survival Indices and Body Weight.

Parameter	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
Implantation sites/dam	17.1	16.8	17.2	16.9
Total pups/dam on day 1	15.5	15.5	15.9	15.0
Viable pups/dam on day 1	15.5	15.7* (15.0)	15.8	14.8
Post-implantation survival index, %	91 (15.5/17.1)	92 (15.5/16.8)	92 (15.9/17.2)	89 (15.0/16.9)
Birth Index, %	91 (15.5/17.1)	89 (15.0/16.8)	92 (15.8/17.2)	88 (14.8/16.9)
Live Birth Index, %	100 (15.5/15.5)	97 (15.0/15.5)	99 (15.8/15.9)	99 (14.8/15.0)
Viability Index, % day 4 postpartum	99 (15.4/15.5)	98 (14.6/15.0)	99 (15.6/15.8)	98 (14.5/14.8)
Lactation Index, % on day 25 postpartum	99 (8.0/8.0)	99 (8.0/8.0)	95 (7.6/8)	86* (6.9/8.0)
Body weights, g (M/F)				
-day 1	6.8/6.4	6.7/6.4	6.7/6.2	6.8/6.4
-day 4 before cull	9.4/8.9	9.2/8.7	9.0/8.6	9.4/8.9
-day 4 after cull	9.5/9.0	9.3/8.9	9.0/8.7	9.5/9.0
-day 25 after cull	79.7/75.4	77.7/74.3	77.3/72.3	74.9/70.9
Male/Female Pups (Ratio)				
-Total on day 1 postpartum	186/187	190/182	205/177	147/168
-# Alive on day 1 postpartum	185/187	185/175	204/175	144/167
-# Alive on day 4 before cull	184/186	181/170	203/172	141/164
-# Alive on day 4 after cull	95/97	92/92	97/95	82/86
-# Alive on day 25 after cull	94/97	91/92	88/86	70/74

* Table 7 containing Group mean litter sizes (F_1) on Page 64, Volume 23 of 218 (or Item 5 Volume 14) appears to contain numerous inaccuracies. For example, there appears to be an error with regard to number of viable F_1 pups/dam at 5 mg/kg/day on day 1 before cull. The total number and viable number of F_1 pups/dam are listed as 15.5 and 15.7, respectively. It is expected that the number of viable pups/dam would be ≤ total number of pups/dam.

Necropsy Findings for F_1 Offspring that Died Prior to Scheduled Termination or were Sacrificed at Scheduled Termination after Week. Expressed as % Incidence/Number of Litters. Data expressed as % Incidence/number of litters.

Parameter	Offspring Which Died Before Termination				Offspring at Termination			
Dose, mg/kg/day	0	5	20	75	0	5	20	75
Offspring/Litters	2/2	12/5	22/6	29/7	151/24	140/23	132/22	103/18
M/F Offspring	2/0	5/7	11/11	16/13	74/77	70/70	67/65	49/54
Swollen area on 1 forepaw containing firm pale material	0	0	0	0	0	0	0	1.0%/1
All digits cyanosed	0	0	0	3.4%/1	0	0	0	0
Cecum enlarged	0	0	0	3.4%/1	0	0	0	0
Unilateral hydronephrosis	0	0	0	10.3%/2	8.6%/7	2.9%/7	5.3%/4	3.9%/3
Bilateral hydronephrosis	0	0	0	3.4%/1	0	0	0.8%/1	1.9%/1
Pedunculate red body attached to testicular fat	0	0	0	0	0	0	0	2.0%/1

6. **F_1 Generation Selected for Assessment of Sexual Maturity, Fertility, and Reproductive Performance**: Body weight gains for F_1 male offspring from weeks 5 to 14 were unaffected by treatment of F_0 dams; although, absolute body weights of F_1 males at 75 mg/kg/day tended to be slightly lower than that of controls. Body weight gains of F_1 female offspring from weeks 5 to 9 and from days 0 to 20 of gestation were unaffected by treatment of F_0 dams; although, absolute body weights of F_1 females at 75 mg/kg/day tended to be slightly lower than that of controls. Duration of the mating periods for F_1 offspring were unaffected by treatment of F_0 dams. Fertility and mating performance in the F_1 generation were unaffected by treatment of F_0 females. Necropsy examination of F_1 dams on day 20 of gestation revealed no macroscopic abnormalities. There were no treatment-related effects on numbers of corpora lutea/dam, or resorptions/dam. Implantations/dam and viable fetuses/dam were slightly decreased at 25 mg/kg/day. There were no treatment-related effects on pre-implantation loss, post-implantation loss, fetal weight, or placental weight. External examination of F_2 fetuses found no treatment-related effects. Necropsy examination of F_1 male rats found no treatment-related changes.

Mating Performance and Fertility of the F_1 Generation

Parameter	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
# Males/Females paired	20/20	20/20	20/20	20/20
#Males mating	20	20	20	20
#Females mating	20	20	20	20
#Males producing pregnancy	19	20	19	20
#Females achieving pregnancy	19	20	19	20
% Males Mating	100	100	100	100
% Females Mating	100	100	100	100
Males-Conception Rate, %	95	100	95	100
Females-Conception Rate, %	95	100	95	100
Males-Fertility Rate, %	95	100	95	100
Females-Fertility Rate, %	95	100	95	100

Group mean litter data for F_1 pregnant female rats sacrificed on day 20 of gestation.

Parameter	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
Number of pregnant animals	19	19	19	20
Corpora lutea/dam	18.3	17.3	17.9	18.8
Implantations/dam	16.3	16.4	16.8	16.4
Viable fetuses/dam	15.6	15.6	16.2	15.5
Resorptions				
-early	0.63	0.79	0.68	0.90
-late	0	0	0	0
-total	0.63	0.79	0.68	0.90
Pre-implantation loss, %	11.5	6.0	6.4	13.0
Post-implantation loss, %	3.9	4.8	4.1	5.5
Fetal weight, g	3.71	3.71	3.74	3.77
Placental weight, g	0.51	0.52	0.52	0.54

External Examination of F_2 Fetuses. Data expressed as %Incidence/number of litters.

Parameter	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	75 mg/kg/day
Fetuses/Litters	297/19	297/19	307/19	309/20
Male/Female	155/142	155/142	153/154	165/144
Pale Fetus	0	0	0	0.3%/1
Body length reduced	0	0.3%/1	0	1.0%/1
Bilateral fore-limb flexure	0.3%/1	0	0	1.3%/1
Clotted blood around placenta	0	0	0	0.6%/1

In an intravenous Segment III perinatal and postnatal development study, pregnant female Sprague-Dawley rats received tinzaparin at doses of 0, 5, 20, and 75 mg/kg/day from days 17 to 20 of gestation and days 1 to 25 postpartum. Tinzaparin at intravenous doses ≤75 mg/kg/day had no effects on perinatal and postnatal development. Mortality of F_0 dams occurred at doses of 20 and 75 mg/kg/day. Internal examinations of F_0 dams revealed signs of hemorrhage. Body weight gains and food consumption for F_0 dams at 75 mg/kg/day from days 17 to 21 of gestation were impaired. Body weight gains and food consumption for F_0 dams from days 1 to 25 postpartum were unaffected by treatment. The lactation indexes at doses of 20 and 75 mg/kg/day on day 25 postpartum were reduced to 95 and 86%, respectively; however, these effects appeared to be directly linked to maternal toxicity. Body weight gains for male and female F_1 pups from days 1 to 25 postpartum were reduced to 93.4 and 93.5% of the control, respectively; although, maternal toxicity was evident at these doses. Body weight gains of F_1 pups in treatment groups after day 25 postpartum were comparable to the control group. The male to female pup ratio at 75 mg/kg/day prior to culling on day 4 (1:1.14 to 1:1.16) appeared to differ from the ratio observed for the control (1:1.01).

Segment III: Perinatal and Postnatal Development Study in the Rat (LSR Report # 88/NLP030/245).

Conducting Lab: [redacted]

Dates of Conduct: Initiated 7/15/87, completed 1/26/88.

GLP Statement: It is stated that the study was conducted in accordance with the internationally recognized GLPs including those of the Japanese Ministry of Health and Welfare. It is implied that it is in accordance with the FDAs' GLPs.

Chemical: LHN-1, lot #s BN 100487, 120487, and Heparin lot # 150487; tested periodically for stability/purity and concentration.

Animals: Pregnant Rats, Sprague-Dawley, [redacted] 22 females (9-11wks old), in each treatment group.

Doses: 0 (control vehicle), 4, 10, and 25 mg/kg/day LHN-1, and Heparin at 12.5 mg/kg/d, given subcutaneously in 0.5 mL/kg volume, at different sites, once daily. The doses were selected on the basis of a dose-range finding study (LSR report #87/NLP024/729), the results of which were not provided.

Methods: The pregnant rats received control vehicle or the test agent once daily on days 15 of gestation through weaning (day 21 postpartum). The rats were observed daily for mortality and morbidity. They were weighed on days 0, 6, 10, and daily from day 15 though 21 of gestation and daily up to day 25 postpartum; food consumption was recorded on days 0, 3, 6, 10, 15, 18, and 21 of gestation, days 1, 4, 7, 11, 18, 21, and 25 of lactation. The gestation length was recorded. At weaning, the dams (F_0) were killed and necropsied; viscera and traces of implantation sites were examined. At birth, the litters (F_1 offspring) were examined for signs of any abnormalities; the pups were weighed, sized (length), and sex-ratio determined. The physical development of at least eight pups (4M, 4F) from each litter was recorded in terms of the day of positive finding: pinna unfolding, tooth eruption, eye opening, testes descent, and vaginal opening. The pups were subjected to a number of tests to measure their auditory, visual, olfactory, and neuromuscular functions on appropriate days.

The reproductive performance of 5-weeks old pups (instead of normal 9-10 weeks of age) was assessed by mating randomly selected one male with one female pup/litter/ treatment group avoiding mating between siblings. On day 20 of gestation, female (F_1) rats were necropsied and the uterine horns were examined for the number of corpora lutea, implantation sites, number of live/dead fetuses etc. A number of indices: mating, fertility, gestation, birth, vitality and weaning were recorded.

Results: Of significance was a finding of hematomas in 3 of 22 and 4 of 22 rats in the high dose LHN-1 and Heparin groups. One female rat in the Heparin group had to be killed due to a large hematoma at the site of injection.

Throughout gestation, the body weight gains of all treated rats and their food consumption in the mid and high dose groups remained marginally lower than controls.

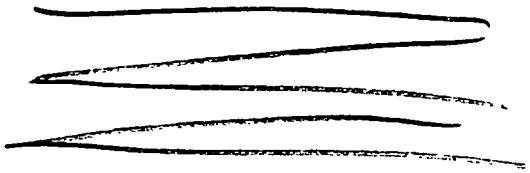
The length of gestation was similar (22-23) across all treatment groups. The number of live/dead fetuses, corpora lutea etc. in the control and treatment groups were not different. LHN-1 did not affect any of the variables: litter size, postnatal mortality, the pup body weight (slight [11%] reduction in the high dose LHN-1 group), sex ratio and the pup length.

During lactation, the growth of the pups was also normal. The development of the offspring (assessed by auditory, visual and neuromuscular function) born to the treated rats was also normal. LHN-1 did not affect significantly the development of the offspring (F_1) or their reproductive performance.

In summary, the treatment of the pregnant rats with LHN-1 at doses of up to 25 mg/kg/d or Heparin at 12.5 mg/kg/d on days 15 of gestation through weaning produced some hematomas/hemorrhages at injection sites, but did not produce any toxic effects on the pregnant mother and general postnatal growth and development of the offspring during lactation or its reproductive performance at doses of up to 25 mg/kg/d.

Addendum:

Testing Laboratory:



Drug Batch: Tinzaparin bulk drug batch F668A — anti-Factor Xa IU/mg) was supplied as solutions containing sodium metabisulfite. The following lot numbers were used: BN100487, BN110487, and BN120487.

Doses: Doses were equivalent to 0, 300, 700, and 1800 anti-Factor Xa IU/kg/day, respectively.

Mortality: One female rat receiving heparin was sacrificed on day 22 postpartum. Internal examination found a large subcutaneous hematoma.

Body Weight and Food Consumption of F_0 Dams: Body weight gain and food consumption of F_0 dams from days 17 to 21 of gestation and days 1 to 25 postpartum were unaffected by treatment. Body weights of female controls on days 17 and 21 of gestation were 326 and 380 g, respectively. Body weight gains for female rats at 4, 10, and 25 mg/kg/day from days 17 to 21 of gestation were 95.6, 98.4, 96.9, and 99.4% of the control, respectively. Body weight gain for female rats receiving heparin from days 17 to 21 of gestation was 99.4% of the control. Body weights of female controls on days 1 and 25 postpartum were 271 and 292 g, respectively. Body weight gains of female rats at 4, 10, and 25 mg/kg/day from days 1 to 25 postpartum were 106.7, 137.4, and 110.3% of the control, respectively. Body weight gain of female rats receiving heparin from days 1 to 25 postpartum was 106.7% of the control.

Litter Data for F_0 Female Rats Allowed to Deliver Their Offspring: Gestation length and index were unaffected by treatment. F_1 pup viability was unaffected by treatment. Body weight gains for F_1 pups at 4, 10, and 25 mg/kg/day from days 1 to 25 postpartum were decreased to 93.1, 91.6, and 87.9% of the control, respectively. Body weight gain for F_1 pups receiving heparin from days 1 to 25 postpartum was decreased to 88.8% of control. Body weight gain for F_1 pups from weeks 5 to 8 were unaffected by treatment. The following parameters for F_1 pups were unaffected by treatment: sex ratios, physical development, auditory and visual responses, locomotor activity, water maze swimming times, and neuromuscular function. Necropsy of F_0 dams after day 25 postpartum found increased incidences of hematoma and hemorrhage at injection sites.

Group Mean Litter Sizes of F_0 Dams Allowed to Litter.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
Implantation Sites	14.8	15.0	15.0	15.2	15.0
Total fetuses/dam on day 1	14.0	14.1	13.5	14.1	14.3
Viable fetuses/dam on day 1	13.6	14.0	13.3	14.0	14.2
Post-implantation survival index, %	95 (14.0/14.8)	94 (14.1/15.0)	90 (13.5/15.0)	92 (14.1/15.2)	95 (14.3/15.0)
Live birth index, %	97 (13.6/14.0)	99 (14.0/14.1)	99 (13.3/13.5)	99 (14.0/14.1)	99 (14.2/14.3)
Viability Index on day 4, %	94 (12.8/13.6)	97 (13.5/14.0)	84 (11.2/13.3)	91 (12.8/14.0)	96 (13.7/14.2)
Lactation Index on day 25 postpartum, %	98 (7.8/7.9)	99 (7.7/7.8)	95 (7.4/7.5)	97 (7.6/7.8)	97 (7.8/8.0)
F_1 pup body weight					
Day 1 postpartum	5.8	6.0	5.9	5.9	5.9
Day 25 postpartum	62.6	60.7	58.8	56.7	57.2

Necropsy Examination of F_0 Females After Day 25 Postpartum.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
Hematoma(e) at ≥1 injection sites	2	5	5	11	14
Hemorrhagic at ≥1 injection sites	0	3	4	3	7

Fertility and Reproductive Performance of F_1 Offspring: Body weight gains for F_1 male rats from weeks 5 to 14 were unaffected by treatment. Body weight gains for F_1 female rats from weeks 5 to 9 and during gestation were unaffected by treatment. The pre-coital interval and fertility and mating performance for the F_1 generation were unaffected by treatment. For F_1 female rats sacrificed on day 20 of gestation, the number of corpora lutea/dam, number of implantation sites/dam, number of viable pups/dam, number of resorptions/dam, pre-implantation loss, post-implantation loss, fetal body weight, and placental weight were unaffected by treatment.

Group Mean Litter Data for F_1 Female Rats Killed on Day 20 of Gestation.

Parameter	0 mg/kg/day	4 mg/kg/day	10 mg/kg/day	25 mg/kg/day	Heparin
#Pregnant F_1 rats	19	20	20	20	20
Corpora lutea/dam	16.4	17.4	17.3	16.4	17.5
Implantation sites/dam	14.0	15.0	15.6	15.2	14.9
Viable F_2 pups/dam	13.3	14.1	14.8	14.1	14.2
Resorptions					
-early	0.63	0.8	0.65	0.90	0.70
-late	0.05	0.1	0.20	0.25	0.05
-total	0.68	0.9	0.85	1.15	0.75
Pre-implantation loss, %	14.7	14.6	9.6	7.6	14.9
Post-implantation loss, %	4.9	6.0	5.4	7.6	5.0
Fetal weight, g	3.35	3.39	3.38	3.41	3.40
Placental weight, g	0.54	0.53	0.53	0.51	0.52

In a subcutaneous Segment III perinatal and postnatal development study, pregnant female rats received tinzaparin at doses of 4, 10, and 25 mg/kg/day from day 15 of gestation through day 21 postpartum. Heparin at a subcutaneous dose of 12.5 mg/kg/day was administered to a group of pregnant female rats as a comparator. Tinzaparin at subcutaneous doses ≤25 mg/kg/day had no effects on perinatal and postnatal development in rats.

Genotoxicity

Assessment of Mutagenic Potential in Histidine Auxotrophs of Samlmonella Typhimurium (Ames Test) (LSR Report No. 85/NLP006/462).

Methods: In a study conducted by [redacted] in compliance with "OECD" Guidelines, concentrations of LHN-1, Batch F85010, varying from 50 to 5000 microgram per plate were evaluated against Salmonella typhimurium strains TA 98, TA 100, TA 1535, and TA 1537, with and without metabolic activation with S-9. Statistical analyses were not done.

Results: No increase in revertants was observed with any concentration of LHN-1 against any of the strains, with or without metabolic activation, vs. distilled water controls. The positive controls benzopyrene, 2-nitroflourene, sodium azide, and 9-aminoacridine, and 2-aminoanthracene were each markedly active against one or more of the test strains in the appropriate system.

Addendum:

Study Completed: July 30, 1985

Drug Batch: Tinzaparin bulk drug batch F85010 [redacted] anti-Factor Xa IU/mg).

Tinzaparin was not mutagenic in the Ames Test with Salmonella typhimurium tester strains TA 1535, TA 1537, TA 100, and TA 98. This study is complementary to LSR Report No. 88/NLP081/0649, which reports no mutagenic activity of tinzaparin with Escherichia coli strains WP2 and WP2 uvrA. Strains WP2 and WP2 uvrA detect mutations at A-T sites.

Assessment of Mutagenic Potential of Trytophan Auxotrophs of Escherichia Coli (LSR Report No. 88/NLP081/0649).

Testing Laboratory: [redacted]

Date Started: August 23, 1988

Date Completed: October 28, 1988

GLP Compliance: Statements of compliance with GLP Regulations and the Quality Assurance Unit were included.

Drug Batch: Tinzaparin, Batch no. F547 (— anti-Factor Xa IU/mg).

Methods: The mutagenic activity of tinzaparin was assessed with Escherichia coli strains WP2 and WP2 uvrA using the pour-plate technique. A S-9 metabolic activation mix, prepared from livers of male CD rats treated with a single intraperitoneal injection of Aroclor 1254 at 500 mg/kg, was used in these studies. A preliminary toxicity assay using tinzaparin levels ranging from 2.5 µg to 5 mg/plate was performed to identify appropriate concentrations for the mutagenicity assay. Positive controls were 2-aminoanthracene (2 µg/plate; requires metabolic activation) in the presence or absence of metabolic activation and N-ethyl-N'-nitro-N-nitrosoguandine (2 µg/plate for WP2 uvrA and 20 µg/plate for WP2) in the absence of metabolic activation. Criteria for a positive response were not described.

Results: For the mutagenicity assays with WP2 and WP2 uvrA, tinzaparin was used at levels of 50, 158, 500, 1590, and 5000 µg/plate. Tinzaparin at levels ≤5000 µg/plate was not mutagenic with Escherichia coli strains WP2 and WP2 uvrA. Positive controls produced expected responses.

Tinzaparin at levels ≤5000 µg/plate was not mutagenic with Escherichia coli strains WP2 and WP2 uvrA.

Assessment of Clastogenic Action on Bone Marrow Erythrocytes in the Micronucleus Test (85/NLP007/726).

Methods: In a study conducted in Sept. - Oct., 1985 by ——— mice of the CD-strain were injected I.V. once with 10, 50, or 250 mg/kg of LHN-1, Batch F85010, dissolved in saline. These dosages were deemed appropriate by prior toxicity tests where deaths occurred at 500 mg/kg and above. A single oral dose of 30 mg/kg of chlorambucil was used as the positive control.

Results:

The mean incidence of micronucleated polychromatic erythrocytes in drug treated mice sacrificed 24, 48, or 72 hours post drug administration was in all cases similar to controls and not statistically different. The positive control chlorambucil however produced statistically significant ($p < 0.01$) increases in micronucleated polychromatic erythrocytes 24 hours post dosing.

In addition, the ratio of polychromatic (immature) erythrocytes to mature erythrocytes 24 and 72 hours post drug was close to 1 in both LHN-1 groups and controls, thus indicating no LHN-1 induced alteration of erythrocyte cell division. On the other hand the positive control chlorambucil reduced the ratio to 0.7, suggesting inhibition of erythrocyte cell division.

Addendum:

Drug Batch: Tinzaparin bulk drug batch F8501([redacted] anti-Factor Xa IU/mg).

Tinzaparin possessed no genotoxic activity in the mouse micronucleus assay.

In Vitro Assessment of the Clastogenic Activity of LHN-1 in Cultured Human Lymphocytes (LSR Report No. 87/NLP032/734).

Methods: In a study conducted Sept. 1987 by [redacted] according to FDA GLPs, 1000, 2000, and 4000 microgram/ml of LHN-1 (5000 mcg/ml was earlier shown to be cytotoxic), with and without S-9, was compared to distilled water or positive controls cyclophosphamide or chlorambucil (the former requiring biotransformation but the latter being a direct mutagen).

Results: None of the concentrations of LHN-1, with or without S-9, induced a significant ($p = >0.05$) increase in incidence of aberrant metaphases, whereas each pos. control was significantly active ($p = <0.001$). For example, the incidence of aberrant metaphases was 1% in the control group without S-9 compared to 1.7, 2.0 and 2.7% with corresponding low, mid, and high concentrations and 1.3% in the control group with S-9 vs 1.7, 2.0, and 3.0% in corresponding L, M and H test groups. In contrast, the incidence with cyclophosphamide, with S-9 was 19.7% and the incidence with chlorambucil, without S-9, was 88.9%.

Addendum: Numbers reported in review are total aberrations.

Drug Batch: Tinzaparin bulk drug batch F668A ([redacted] anti-Factor Xa IU/mg).

Tinzaparin possessed no clastogenic activity in the human lymphocyte chromosomal aberration assay.

Investigation of Mutagenic Activity at the HGPRT Locus in a Chinese Hamster V79 Cell Mutation System (LSR Report No. 87/NLP033/768).

Methods: In a study assessing the ability of LHN-1 (Lot # 190487) to induce mutation in Chinese hamster (V79) cells at the hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) locus, concentrations of 312.5 to 5000 microgram/ml were selected based on prior testing. Negative controls included distilled water and sodium metabisulphite, a constituent of the LHN-1 preparation; the positive controls included ethyl

methanesulphonate (EMS), a direct acting mutagen, and 7, 12-dimethyl benzanthracene (DMBA), a mutagen that requires metabolic activity for optimum expression. The study was conducted by [redacted] according to FDA GLPs.

Results: In the first of 2 assays, LHN-1 did not increase the frequency of mutations significantly compared to both negative controls, with or without S-9; in the second assay however, mutation frequencies were slightly increased, both with and without S-9, compared to either negative control. In a supplemental third assay however, no increase in mutation frequency was detected in any test group, with or without activation, thus confirming the findings of the first assay. The incidence of mutations with either positive control markedly exceeded both negative control groups as well as all test responses, including those slightly elevated at the second assay. Statistical analyses were not made.

Addendum:

Study Completed: March 2, 1988

Drug Batch: Tinzaparin bulk drug batch F668A [redacted] anti-Factor Xa IU/mg).

Tinzaparin was apparently not mutagenic at the HGPRT locus in Chinese hamster V79 cells; although, results were complicated by significant variations in the mutation frequencies for the vehicle controls of the three separate tests.

Special Toxicity Studies

Tinazparin and USP Heparin: Active Anaphylaxis in the Guinea Pig (LSR Report No. 88/NLP060/0402 and First Amendment LSR Report No. 92/NLP060/0344).

Testing Laboratory: [redacted]

Date Started: January 7, 1988

Date Completed: November 15, 1988; First Amendment April 29, 1992

GLP Compliance: Statements of compliance with GLP Regulations and the Quality Assurance Unit were included.

Animals: Male Dunkin-Hartley guinea pigs were used in this study. On day that animals were received, the body weight range was 224-310 g.

Drug Batch: Tinzaparin, Lot No. F682X [redacted] anti-Factor Xa IU/mg).

Methods: The ability of tinzaparin to induce active anaphylaxis was assessed in male Dunkin-Hartley guinea pigs. USP Heparin was used as a comparator. The sponsor's dose selection was a based upon a preliminary study in which guinea pigs were treated with tinzaparin at doses ranging from 1 to 120 mg/kg or USP Heparin at doses ranging from 0.5 to 65 mg/kg. Following the first treatment, one animal that received tinzaparin at 120 mg/kg and two animals that received heparin at 65 mg/kg were observed with pallor and decreased motor activity, and subsequently sacrificed in a moribund condition. All remaining animals survived to challenge. In the present study, guinea pigs received either tinzaparin, USP Heparin, the positive control, bovine serum albumin (BSA), or saline, mixed with an equal volume of Freund's Complete Adjuvant (FCA), to obtain a homogeneous emulsion. Treatments are shown in the table below. Each animal received two subcutaneous injections, administered one week apart, in the dorsal skin between the shoulder blades. The dose volume was 1 mL/kg. Two weeks after the last treatment, guinea pigs received an intravenous challenge dose of 10 mg of either the test material (800 anti-Factor Xa IU), which was used for sensitization, or BSA into the peripheral ear vein. The two control groups, previously treated with saline/FCA, was challenged with either tinzaparin or USP Heparin. Animals were monitored for a 3 hr period following challenge and all treatment-related changes were recorded. Animals surviving the challenge were observed 24 hr later.

Main Study Design

Group	Treatment	Dose, mg/kg	# Animals
1	Saline	0	2
2	Saline	0	2
3	BSA	1.0	4
4	Tinzaparin	2.0 (154 IU/kg)	4
5	Tinzaparin	20.0 (1540 IU/kg)	4
6	Tinzaparin	60.0 (4600 IU/kg)	4
7	USP Heparin	1.0	4
8	USP Heparin	10.0	4
9	USP Heparin	30.0	4

Results: Following the first treatment, all animals that received heparin at 30 mg/kg were observed with signs of pallor and lethargy and subsequently sacrificed in a moribund condition. Following the second treatment, one animal that received tinzaparin at 60 mg/kg was observed with signs of pallor and lethargy and subsequently sacrificed in a moribund condition. Following challenge, all animals treated with BSA displayed signs of anaphylaxis, which consisted of respiratory gasping, violent convulsions, and death within 3 min. Animals treated with saline and subsequently challenged with tinzaparin or heparin displayed no signs of anaphylaxis. None of the animals treated with tinzaparin or USP heparin displayed any signs of anaphylaxis.

Treatment of male Dunkin-Hartley guinea pigs with tinzaparin did not induce any signs of anaphylaxis.

Tinzaparin and USP Heparin: Detection of Anaphylactic Antibodies in the Guinea Pig Using a Passive Cutaneous Anaphylaxis Assay (LSR Report No. 88/NLP061/0401).

Testing Laboratory: [redacted]

Date Started: January 21, 1988

Date Completed: November 15, 1988

GLP Compliance: Statements of compliance with GLP Regulations and the Quality Assurance Unit were included.

Animals: Male Dunkin-Hartley guinea pigs were used in this study. On the day after arrival, body weights ranged from 203 to 312 g.

Drug Batch: Tinzaparin, Lot No. F682X [redacted] anti-Factor Xa IU/mg).

Methods: The ability of tinzaparin to stimulate the production of anaphylactic antibodies was assessed in male Dunkin-Hartley guinea pigs using the passive cutaneous anaphylaxis assay. USP Heparin was used as a comparator. The sponsor's dose selection was a based upon a preliminary study in which guinea pigs were treated with tinzaparin at doses ranging from 1 to 62.5 mg/kg (100, 400, 1900, or 4800 anti-Factor Xa IU/kg) or USP Heparin at doses ranging from 0.5 to 31.25 mg/kg. After the first treatment, one animal that received tinzaparin at 62.5 mg/kg was observed with pallor and lethargy and subsequently sacrificed. In the present study, guinea pigs received either tinzaparin, USP Heparin, the positive control, bovine serum albumin (BSA), or saline, mixed with an equal volume of Freund's Complete Adjuvant (FCA), to obtain a homogeneous emulsion. Treatments are shown in the table below. Each animal received two subcutaneous injections, administered one week apart, in the dorsal skin between the shoulder blades. The dose volume was 1 mL/kg. Two weeks after the last treatment, blood was collected from guinea pigs and serum was isolated for the assay. Serum (i.e., total Ig) was diluted with saline to yield dilutions of 1/2, 1/4, 1/8, 1/16, and 1/32. Serum (i.e., total Ig) from the positive control group was diluted with saline to yield dilutions of 1/4, 1/8, 1/16, 1/32, and 1/64. An aliquot (0.05 mL) of each serum dilution was given by the intradermal route into the shaved back of each animal. Serum from two animals was assayed in each animal. In addition, each animal received two control intradermal injections of saline. Approximately 24 hr after intradermal treatments, guinea pigs received an intravenous challenge with a 0.5 mL solution containing the appropriate test agent and Evan's blue dye at final concentrations of 20 and 40 mg/mL,

respectively. Approximately 30 min after challenge, animals were sacrificed and the blue area at intradermal injection sites was measured. For measurements of anaphylactic IgG_1, serum dilutions were heated at 56°C for 2 hr to inactivate IgE antibody. Serum dilutions were applied to animals as described above and intravenous challenge with the appropriate test agent was given 4 hr later.

Main Study Design

Group	Treatment	Dose, mg/kg	# Animals
1	Saline	0	4
2	Tinzaparin	2.0	4
3	Tinzaparin	20.0	4
4	Tinzaparin	60.0	4
5	USP Heparin	1.0	4
6	USP Heparin	10.0	4
7	USP Heparin	20.0	4
8	BSA	1.0	4

Results: After the first treatment, one animal treated with heparin at 20 mg/kg was observed with signs of pallor and lethargy and subsequently sacrificed. For the positive control, Ig or IgG_1 serum elicited passive cutaneous anaphylactic (PCA) responses at titers ≥1/64. Serum from animals treated with saline followed to elicit PCA responses in assay animals challenged with either tinzaparin or USP Heparin. Serum (Ig or IgG_1) from animals treated with tinzaparin or USP Heparin did not elicit PCA responses in assay animals challenged with the respective test agent.

Tinzaparin produced a negative response in the passive cutaneous anaphylaxis assay with guinea pigs.

Tinzaparin and USP Heparin: Detection of Antibodies Stimulated in the Rabbit Using a Passive Hemagglutination Assay (LSR Report No. 88/NLP062/0403).

Testing Laboratory: [redacted]

Date Started: January 7, 1988

Date Completed: November 25, 1988; First Amendment: April 29, 1992

GLP Compliance: Statements of compliance with GLP Regulations and the Quality Assurance Unit were included.

Animals: Male New Zealand White Rabbits were used in this study. On the day of arrival, body weights ranged from 1.98 to 2.93 kg.

Drug Batch: Tinzaparin, Lot No. F682X [redacted] anti-Factor Xa IU/mg).

Methods: The ability of tinzaparin to stimulate the production of specific antibodies in rabbits was assessed using the passive hemagglutination assay. This assay uses sheep red blood cells, treated to promote passive absorption and subsequent coating with the test material or positive control compound, to detect agglutinating antibodies present in the study animal's serum. USP Heparin was used as a comparator. Rabbits received either tinzaparin, USP Heparin, the positive control, bovine serum albumin (BSA), or saline, mixed with an equal volume of Freund's Complete Adjuvant (FCA), to obtain a homogeneous emulsion. Treatments are shown in the table below. Each animal received three subcutaneous injections, administered one week apart, in the dorsal skin between the shoulder blades. The dose volume was 0.5 mL/kg. Five weeks after the last treatment, blood was collected and serum was isolated for use in the passive hemagglutination assay. Results were scored as complete agglutination, no agglutination, and partial agglutination. Results were expressed as the highest dilution of serum giving complete hemagglutination. The sponsor's dose selection was based upon a preliminary study in which rabbits received tinzaparin at doses ranging from 0.9 to 110 mg/kg or USP Heparin at doses ranging from 0.5 to 62 mg/kg. Signs of pallor and lethargy were observed in one animal after the first treatment with the high dose of USP Heparin and in two animals after the second treatment with the high dose of tinzaparin; however, these animals recovered. One of these animals, that received the high dose of tinzaparin, died 32 day after the third treatment; however, death was attributed to a possible infection.

Main Study Design

Group	Treatment	Dose, mg/kg	# Animals
1	Saline	0	5
2	Tinzaparin	5.0 (385 IU/kg)	5
3	Tinzaparin	50.0 (3850 IU/kg)	5
4	Tinzaparin	100.0 (7710 IU/kg)	5
5	USP Heparin	2.5	5
6	USP Heparin	25.0	5
7	USP Heparin	50.0	5
8	BSA	4.0	5

Results: One animal, that received heparin at 50 mg/kg, died 6 days after the first treatment. Serum from rabbits treated with BSA had high titers of specific antibody to this antigen. Serum from animals treated with saline did not produce hemagglutination with either coated or uncoated cells. No specific antibodies to USP Heparin were detected in serum from animals treated with this test material. No agglutinating antibodies to tinzaparin were found in animals treated with compound at 5 or 100 mg/kg. Serum from 3 of 5 animals treated with tinzaparin at 50 mg/kg showed no hemagglutination; however, serum from the remaining 2 animals produced a borderline positive hemagglutination response. Given that no agglutinating antibodies were found with tinzaparin at 5 or 100 mg/kg, the passive hemagglutination response with this test material is considered to be negative.

Serum from rabbits treated with tinzaparin (5, 50, or 100 mg/kg/day) did not produce a specific passive hemagglutination response.

Local Irritation in Rabbits After Intramuscular Injection of Tinzaparin (Study #3785).

Methods: In a study conducted by Novo on June 25, 1985, a group of six rabbits were injected once into the left sacrospinalis muscle with 0.25ml of a 300 mg/ml solution (30%) of LHN-1; the right contralateral muscle was similarly injected with 0.25ml of 0.9% saline. Another group of 6 rabbits was injected once with 1 ml of sterile acetic acid at 0.75% on one side and 6% on the other. One half of the animals were sacrificed 2 days post injection and examined locally both grossly and microscopically; similar examinations were made 7 days post-injection on the remaining animals.

Results:

Macroscopically 2 days post injection, LHN-1 was associated with moderate to strong hemorrhage along the injection canal vs slight hemorrhage with saline; at 7 days a dark narrow hemorrhage zone marked the injection canal in the drug treated vs no sign of damage in the saline control. The size of the damage was too small to measure with either LHN-I or saline. In contrast, both concentrations of acetic acid produced large dark necrotic areas in the muscle, evident both 2 and 7 days post injection.

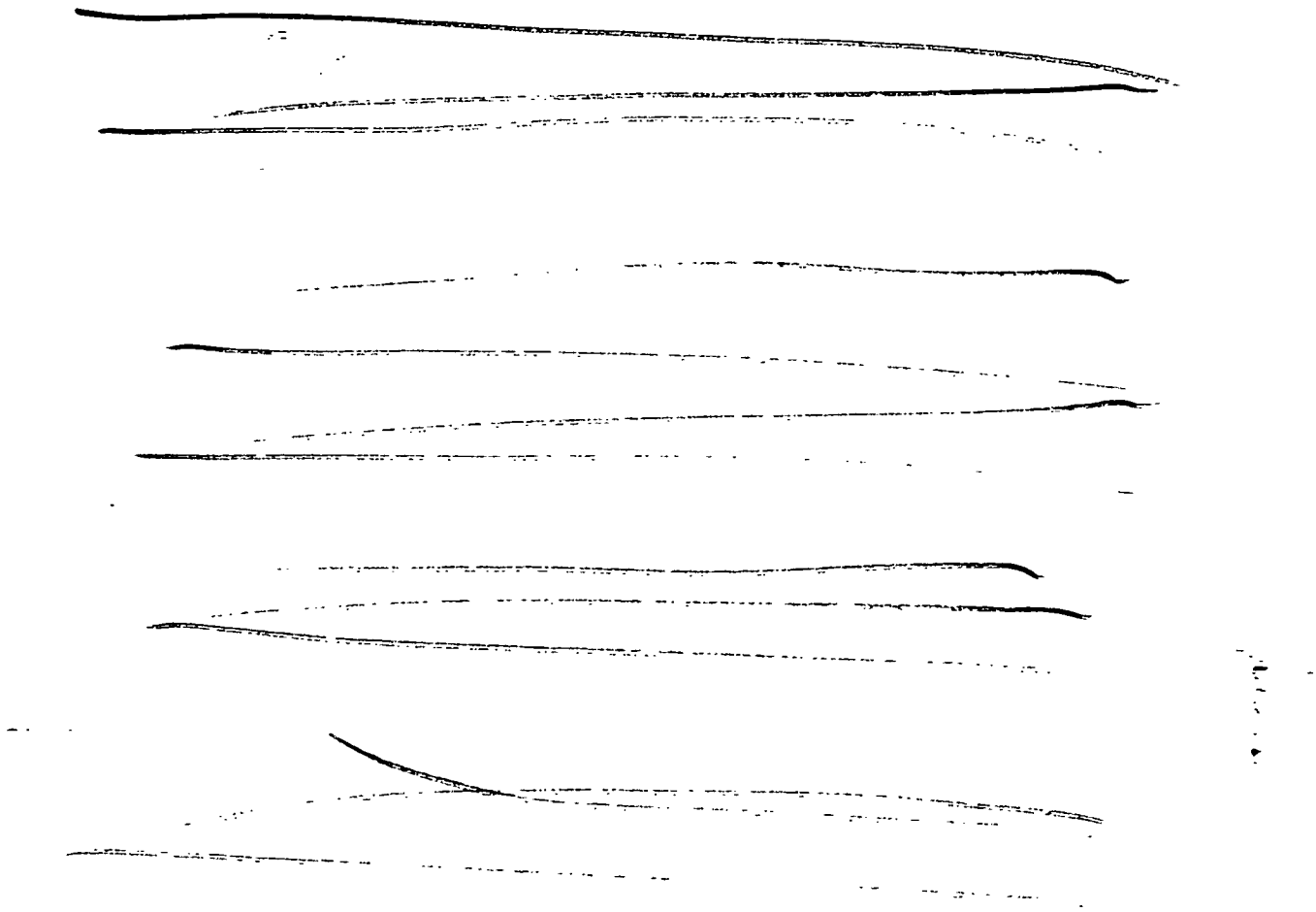
Microscopically, the LHN-1 treated sites showed only slight hemorrhage and edema, a few necrotic muscle fibers, and some inflammatory cell infiltration, particularly at 2 days post drug. Similar but slightly milder lesions were present in saline treated 2 days post drug (saline treated sites not examined 7 days post-drug). The acetic acid treated sites showed large necrotic areas with severe hemorrhage and edema at both examination periods; fibrosis and muscle regeneration were apparent at the second exam time.

It was concluded that LHN-1 produced moderate irritation consistent with the anti-coagulant properties of the drug and that the severity of the irritation was slightly greater than 0.9% saline, but considerately less than either concentration of acetic acid.

Addendum:

Drug Batch: Tinzaparin bulk drug batch F84001 ([redacted]anti-Factor Xa IU/mg).

3 page(s) of revised draft labeling has been redacted from this portion of the review.



SUMMARY AND EVALUATION

Tinzaparin is an enzymatically depolymerized heparin with an average molecular weight of approximately 5000 daltons determined by gel filtration chromatography. The molecular weight distribution of tinzaparin is broad ranging from about 600 (disaccharides) to above 15,000 daltons. Tinzaparin has the same chemical structure as heparin except for the terminal unsaturated hexuronic acid residues ($\Delta_{4,5}$ iduronic acid) produced during the depolymerization process. These unsaturated iduronic residues, which absorb light UV light at 235 nm occur in about two-thirds of the molecules. The in vitro anti-factor Xa activity of tinzaparin is approximately 100 IU per milligram (range of 70 to 120 IU per milligram). Both tinzaparin (0.13-1.54 mg/kg) and heparin (0.07-0.89 mg/kg) were able to inhibit thrombus formation in the rabbit model of experimental thrombosis. Bleeding time was prolonged following administration of either heparin (0.5 and 1 mg/kg) or tinzaparin (1.2 and 2.4 mg/kg). In a stasis model using female Wistar rats, tinzaparin or heparin at doses of 1.25 to 5 mg/kg produced a dose-dependent inhibition of thrombus formation with complete inhibition observed at 5 mg/kg. Tinzaparin at subcutaneous doses of 1.25 to 5 mg/kg had no effects on

bleeding times as assessed by the tail transection technique in female Wistar rats. In contrast, heparin at 5 mg/kg significantly prolonged bleeding times. Administration of heparin by the intravenous route at a dose of 3 mg/kg to male Wistar rats produced increases of APTT and anti-factor Xa activity, which were both neutralized by protamine sulfate at 3.5 mg/kg. In contrast, tinzaparin administered by the intravenous route at 7.2 mg/kg produced an increase of APTT, which was neutralized by protamine sulfate at 5 mg/kg; however, increases of antithrombotic and anti-factor Xa activities were neutralized by protamine sulfate at 13 mg/kg. For tinzaparin, anti-factor Xa activity and antithrombotic effects were neutralized by a significantly higher dose of protamine sulfate as compared to hemorrhagic effects suggesting a clear separation between antithrombotic and hemorrhagic effects. In summary, tinzaparin, like unfractionated heparin, is a potent co-inhibitor of several activated coagulation factors, particularly factor Xa. Tinzaparin has a higher ratio of anti-factor Xa activity to anti-factor IIa activity than unfractionated heparin. Tinzaparin, most importantly, possesses a distinct advantage over unfractionated heparin in that it has more predictable pharmacokinetic behavior and can be administered once daily as a subcutaneous injection without the need for therapeutic monitoring of anticoagulant activity.

Tinzaparin is indicated for the initial treatment of acute symptomatic deep vein thrombosis with and without pulmonary embolism when administered in conjunction with warfarin sodium.

The sponsor has submitted the preclinical pharmacology and toxicology studies in support of tinzaparin (INNOHEP®) as follows: pharmacology; absorption, distribution, metabolism, and excretion studies in rats, rabbits, and dogs; acute toxicity studies in mice and rats; a 4-week intravenous toxicity in rats; a 26-week intravenous toxicity study in rats; a 1-year subcutaneous toxicity study in rats; a 4-week intravenous toxicity study in dogs; a 1-year intravenous toxicity study in dogs; a 1-year subcutaneous toxicity study in dogs; reproductive toxicology studies consisting of Segment I intravenous and subcutaneous fertility and reproductive performance studies in rats, Segment II intravenous and subcutaneous tetratology studies in rats and rabbits, and Segment III intravenous and subcutaneous perinatal and postnatal development studies in rats; genotoxicity studies consisting of in vitro bacterial reverse mutation assays, an in vitro lymphocyte chromosomal aberration assay, an in vitro Chinese hamster ovary cell forward mutation assay (CHO/HGPRT), and an in vivo mouse micronucleus test; and special toxicity studies consisting of an active anaphylaxis assay in guinea pigs, a passive cutaneous anaphylaxis assay in guinea pigs, passive hemagglutination assay in rabbits, and a local irritation assay in rabbits following intramuscular injection.

The absorption, distribution, metabolism, and excretion of tinzaparin were assessed in rats, rabbits, and dogs. Following administration of ^{3}H-tinzaparin by the subcutaneous or intravenous route at doses of 1 or 4 mg/kg to rats or dogs, plasma AUC values increased in a dose proportional manner. For rats following subcutaneous administration of 1 or 4 mg/kg, C_{max} values based upon drug-related radioactivity were 0.9 and 4.1 µg/mL, respectively. T_{max} and T½ values both ranged from 0.50 to 0.75 hr.

For rats following intravenous administration of 1 or 4 mg/kg, C_{max} values were 2.8 and 12.7 µg/mL, respectively. T½ values ranged from 0.70 to 0.91 hr. For dogs following subcutaneous administration of 1 or 4 mg/kg, C_{max} values based upon drug-related radioactivity were 0.86 and 4.50 µg/mL, respectively. T_{max} and T½ values both ranged from 1.31 to 1.67 hr. For dogs following intravenous administration of 1 or 4 mg/kg, C_{max} values were 5.63 and 22.26 µg/mL, respectively. T½ values ranged from 0.88 to 1.14 hr. Following subcutaneous administration of tinzaparin at 4 or 25 mg/kg to rabbits, based upon either plasma levels of drug-related radioactivity, anti-Factor Xa activity or anti-Factor IIa activity, AUC and C_{max} values increased in a dose proportional manner. Bioavailability of tinzaparin administered by the subcutaneous route to rats or dogs, as assessed by plasma drug-related radioactivity was approximately 100%. In contrast, bioavailability, as assessed by anti-Factor Xa activity, was approximately 70%. Clearance of tinzaparin in rats (0.65-0.75 L/hr/kg), based upon plasma drug-related radioactivity or anti-Factor Xa activity was between the glomerular filtration (0.2 L/hr/kg) and renal plasma flow (1.3 L/hr/kg). Clearance of tinzaparin in dogs (0.21-0.25 L/hr/kg), based upon drug-related radioactivity was comparable to the glomerular filtration rate (0.21 L plasma/hr/kg); however, clearance, based upon anti-Factor Xa activity, was significantly less than the glomerular filtration rate. Clearance following intravenous administration of 4500 IU tinzaparin to healthy human volunteers was 1.7 L/hr (i.e., approximately 0.034 L/hr/kg for a 50-kg person) and the primary route of elimination was renal. For rats, rabbits, and dogs, volume of distribution values for tinzaparin, based upon plasma levels of anti-Factor Xa and anti-Factor IIa activities, volume of distribution values for tinzaparin were approximately equivalent to blood volume; however, volume of distribution values, based upon plasma levels of radioactivity, exceeded blood volume suggesting distribution into tissue. For healthy human volunteers, the volume of distribution as indicated by anti-Factor Xa activity was approximately equivalent to the blood volume (i.e., central compartment). Differences in pharmacokinetic parameters based upon plasma drug-related radioactivity and anti-Factor Xa/anti-Factor IIa activity most likely reflect the broad distribution of molecular weights for the components of tinzaparin. Tissue distribution studies in rats following subcutaneous or intravenous administration of ^{3}H-tinzaparin using whole body autoradiography or measurement of tissue radioactivity suggested a widespread distribution of radioactivity. Initially, tissue radioactivity concentrations were highest in the kidney, which was consistent with this organ's primary role in excretion. At later time points, high concentrations of radioactivity were observed in the liver. Studies with pregnant female rats and rabbits suggested that small amounts of drug-related radioactivity could cross the placenta and enter fetal tissues. Studies with lactating female rats indicated that low levels of drug-related radioactivity were excreted into the milk; although, there were negligible levels of radioactivity evident in suckling pups. Chromatographic examination of urine excreted from rats and dogs treated with ^{3}H-tinzaparin indicated no significant metabolism of the test article. Excretion studies in rats and dogs indicated that 70 to 80% of drug-related radioactivity was excreted in the urine. Negligible levels of drug-related radioactivity were excreted in the feces or bile.

The acute toxicity of tinzaparin was examined in mice and rats by the intravenous and subcutaneous routes of administration. Following intravenous administration, the minimum lethal doses in male and female mice were 250 and 500 mg/kg (19200 and 38300 IU/kg), respectively. Following subcutaneous administration, the minimum lethal doses in male and female mice were 500 and 300 mg/kg (38300 and 22000 IU/kg), respectively. Following intravenous administration, the minimum lethal doses in male and female rats were 1600 and 3200 mg/kg (123000 and 245000 IU/kg), respectively. Following subcutaneous administration, the minimum lethal dose in male and female rats was 100 mg/kg/day (7700 IU/kg). The acute toxicity of tinzaparin in mice by the intravenous and subcutaneous routes were relatively similar. In contrast for rats, tinzaparin administered by the subcutaneous route was significantly more toxic than by the intravenous route. Symptoms of acute toxicity included hematoma formation and bleeding at the injection site, anemia, decreased motor activity, unsteady gait, piloerection, and ptosis.

In a 4-week intravenous toxicology study, rats received tinzaparin at doses of 0, 10, 30, and 100 mg/kg/day (equivalent to 0, 800, 2300, and 7700 anti-Factor Xa IU/kg, respectively). Dose related discoloration of the tail occurred in all treatment groups. Bleeding from the injection site was evident at 30 and 100 mg/kg/day. Animals at 30 and 100 mg/kg/day developed hematomas in body areas other than the injection site. Examination of the liver and spleen from animals at 100 mg/kg/day revealed extrahematopoiesis. Hemorrhage in various internal tissues was evident at 100 mg/kg/day. A dose-related reactive lymphoid hyperplasia was evident in all treatment groups. Histopathological findings appear to be consequences of the anticoagulant properties of tinzaparin.

In a 26-week intravenous toxicology study, rats received tinzaparin by the intravenous route at doses of 0, 5, 10, or 25 mg/kg/day (equivalent to 0, 400, 900, and 2200 anti-Factor Xa IU/kg/day, respectively). There were 15 rats/sex/group. An additional 10 rats/sex/group were assigned to the control and 25 mg/kg/day groups for a 4-week recovery period following the 26-week treatment period. The no effect dose was 5 mg/kg/day. Treatment-related mortality appeared to occur at doses of 10 and 25 mg/kg/day. Histopathological changes were observed at low incidences and did not appear to reveal a target organ of toxicity. Progressive nephropathy was observed for 17.9% of rats at 25 mg/kg/day. Evidence of the anticoagulant effect of tinzaparin was observed at 25 mg/kg/day and included erythrocytes and erythrophagocytosis in sinuses for the mandibular and mesenteric lymph nodes, pelvic hemorrhage in the kidney, presence of blood in the lumen of the bronchi, hemorrhage at the injection site,and hemosiderosis in the mesenteric lymph node. At the end of the 4 week recovery period, most of these changes were not observed. Observations at injection sites for tinzaparin-treated groups included bruising, swelling, encrustation, exfoliation, erythema, and prolonged bleeding; however, these changes were not evident by the 3rd week of recovery.

In a one-year subcutaneous toxicology study, rat received tinzaparin at doses of 0, 4, 10, and 25 mg/kg/day (equivalent to 0, 300, 700, and 1800 anti-Factor Xa IU/kg/day, respectively). Heparin at a dose of 12.5 mg/kg/day was used as comparator. The no effect dose was 4 mg/kg/day. Treatment-related mortality occurred for tinzaparin at 10 and 25 mg/kg/day and heparin at 12.5 mg/kg/day. Swellings and hemorrhages at injection sites were observed in all treatment groups. For animals receiving treatment with tinzaparin at 25 mg/kg/day and heparin at 12.5 mg/kg/day and died or were sacrificed in moribund condition during the treatment period, observed changes included occasional dark areas and masses at injection sites and generalized pallor of internal organs (suggestive of blood loss), fluid in the abdomen or thorax and dark gastrointestinal contents. Bone density was decreased for animals receiving heparin, but not for animals receiving tinzparin. Bone ash (% of femur) was decreased for animals receiving tinzaparin at 25 mg/kg/day and heparin at 12.5 mg/kg/day. Following a 6-week recovery period, tissue damage at injection sites had generally resolved.

In a four-week intravenous toxicology study, beagle dogs received tinzaparin at doses of 0, 10, 30, and 50 mg/kg/day (equivalent to 0, 700, 2200, and 3700 anti-Factor Xa IU/kg/day, respectively). Localized irritant and hemorrhagic effects were observed at injection sites for treatment groups. Macroscopic changes (i.e., discoloration, thickening of vein, hemorrhage, edema, and fibrosis in the subcutis) at the injection sites were increased in a dose-related manner regarding incidence and severity for tinzaparin treatment groups.

In a 52-week intravenous toxicology study, beagle dogs received tinzaparin by the intravenous route at doses of 0, 4, 10, or 25 mg/kg/day (equivalent to 0, 300, 900, and 2200 anti-Factor Xa IU/kg/day, respectively). For the control and 25 mg/kg/day groups, there were additional dogs for a 4-week recovery period following the treatment period. The maximum tolerated dose was 25 mg/kg/day. Histopathological changes were primarily confined to the injection sites (i.e., hemorrhage, reactive inflammation/fibrosis) following the 52-week treatment period. Some changes at injection sites (i.e., hemorrhage) were still evident following the 4-week recovery period.

In a 52-week subcutaneous toxicology study, dogs received tinzaparin at doses of 4, 10, and 25 mg/kg/day (equivalent to 0, 300, 700, and 1800 anti-Factor Xa IU/kg/day, respectively). Heparin at 12.5 mg/kg/day was administered by the subcutaneous route to groups of dogs as a comparator. The no effect and maximum tolerated doses for tinzaparin were 4 and 10 mg/kg/day, respectively. Due to excessive mortality for tinzaparin at 25 mg/kg/day and heparin at 12.5 mg/kg/day, doses were reduced to 16.7 mg/kg/day (1200 anti-Factor Xa IU/kg/day) and 5 mg/kg/day, respectively, during week 19 of treatment. Swelling and hemorrhage at injection sites were observed in all treatment groups. Dogs, that died or were sacrificed in moribund condition, were observed with severe hemorrhage, hematomas, and edema at injection sites. Further, high erythroid cell activity was found in these animals, which may have been a compensatory response to blood loss. The target organ of toxicity was the spleen. For tinzaparin at 10 and 25/16.7 mg/kg/day, contraction of the sinuses in the spleen were observed, which was probably related to blood loss.

In an intravenous Segment I fertility and reproductive performance study, rats received tinzaparin at doses of 0, 5, 15, and 50 mg/kg/day (equivalent to 0, 400, 1300, and 4300 anti-Factor Xa IU/kg/day, respectively). Male rats were treated for 71 days prior to pairing, throughout the mating period, and up to termination after necropsy of female rats. Female rats were treated for 15 days prior to pairing, throughout the mating period, and from day 0 to 7 of gestation. Tinzaparin at intravenous doses ≤50 mg/kg/day had no effects on fertility or reproductive performance in rats.

In a subcutaneous Segment I fertility and reproductive performance study, rats received tinzaparin at doses of 0, 4, 10, and 25 mg/kg/day (equivalent to 0, 300, 700, and 1800 anti-Factor Xa IU/kg/day, respectively). Heparin at 12.5 mg/kg/day was administered by the subcutaneous route to groups of rats as a comparator. Male rats were treated for 71 days prior to pairing, throughout the mating period, and up to termination after necropsy of female rats. Female rats were treated for 15 days prior to pairing, throughout the mating period, and continuing to either day 20 of gestation or day 25 postpartum. Tinzaparin at subcutaneous doses ≤25 mg/kg/day had no effects on fertility or reproductive performance in rats.

In an intravenous Segment II teratology study, pregnant female Sprague-Dawley rats received tinzaparin at doses of 0, 5, 20, or 75 mg/kg/day (equivalent to 0, 400, 1700, and 6500 anti-Factor Xa IU/kg/day, respectively) from days 7 to 17 of gestation. Tinzaparin was not teratogenic at intravenous doses ≤75 mg/kg/day. Maternal toxicity was observed at 75 mg/kg/day.

In an intravenous Segment II teratology study, pregnant female Sprague-Dawley rats received tinzaparin at doses of 0, 5, 10, or 25 mg/kg/day (equivalent to 0, 400, 900, and 2200 anti-Factor Xa IU/kg/day, respectively) from days 7 to 17 of gestation. Tinzaparin at intravenous doses ≤25 mg/kg/day was not teratogenic in rats.

In a subcutaneous Segment II teratology study, pregnant female Sprague-Dawley rats received tinzaparin at doses of 0, 4, 10, or 25 mg/kg/day (equivalent to 0, 300, 700, and 1800 anti-Factor Xa IU/kg/day, respectively) from days 7 to 17 of gestation. Heparin was administered by the subcutaneous route at 12.5 mg/kg/day to a group of pregnant female rats as a comparator. Tinzaparin at subcutaneous doses ≤25 mg/kg/day was not teratogenic in rats.

In an intravenous Segment II teratology study, pregnant female rabbits received tinzaparin at doses of 5, 20, or 75 mg/kg/day (equivalent to 0, 400, 1700, and 6500 anti-Factor Xa IU/kg/day, respectively) from days 6 to 18 of gestation. Tinzaparin at intravenous doses ≤75 mg/kg/day was not teratogenic in rabbits.

In a subcutaneous Segment II subcutaneous teratology study, pregnant female rabbits received tinzaparin at doses of 0, 4, 10, or 25 mg/kg/day (equivalent to 0, 300, 700, and 1800 anti-Factor Xa IU/kg/day, respectively) from days 6 to 19 of gestation. Heparin at a subcutaneous dose of 12.5 mg/kg/day was administered to a group of pregnant female rabbits as a comparator. Tinzaparin at subcutaneous doses ≤25 mg/kg/day was not teratogenic in rabbits. Body weight gain was suppressed in all tinzaparin groups as well as the control group during and after the treatment period, which resulted in the sponsor conducting a second teratology study in rabbits, which is described below.

In a subcutaneous Segment II teratology study, pregnant female rabbits received tinzaparin at doses of 0, 2, 8, and 25 mg/kg/day (equivalent to 0, 200, 600, and 1900 anti-Factor Xa IU/kg/day, respectively) from days 6 to 19 of gestation. Tinzaparin at subcutaneous doses ≤25 mg/kg/day was not teratogenic in rabbits.

In an intravenous Segment III perinatal and postnatal development study, pregnant female Sprague-Dawley rats received tinzaparin at doses of 0, 5, 20, and 75 mg/kg/day (equivalent to 0, 400, 1700, and 6500 anti-Factor Xa IU/kg/day, respectively) from days 17 to 20 of gestation and days 1 to 25 postpartum. Tinzaparin at intravenous doses ≤75 mg/kg/day had no effects on perinatal and postnatal development. Maternal toxicity was observed at 75 mg/kg/day.

In a subcutaneous Segment III perinatal and postnatal development study, pregnant female rats received tinzaparin at doses of 4, 10, and 25 mg/kg/day (equivalent to 0, 300, 700, and 1800 anti-Factor Xa IU/kg/day, respectively) from day 15 of gestation through day 21 postpartum. Heparin at a subcutaneous dose of 12.5 mg/kg/day was administered to a group of pregnant female rats as a comparator. Tinzaparin at subcutaneous doses ≤25 mg/kg/day had no effects on perinatal and postnatal development in rats.

Tinzaparin was uniformly negative in a battery of genotoxicity tests that included an in vitro bacterial reverse mutation assay with Salmonella typhimurium tester strains TA1535, TA1537, TA98, and TA100 and Escherichia coli tester strains WP2 and WP2 uvrA: an in vitro human lymphocyte chromosomal aberration assay, an in vitro Chinese hamster ovary forward mutation assay (CHO/HGPRT); and an in vivo mouse micronucleus assay.

The anaphylactic and irritancy potential of tinzaparin was assessed in several assays. Tinzaparin was negative in an assay to assess its ability to induce active anaphylaxis was assessed in male guinea pigs. Tinzaparin produced a negative response in the passive cutaneous anaphylaxis assay with guinea pigs. Serum from rabbits treated with tinzaparin did not produce a specific passive hemagglutination response. Tinzaparin produced moderate irritation in rabbits following intramuscular injection that was consistent with its anticoagulant properties.

In humans, tinzaparin will be administered for prevention of postoperative venous thromboembolism [redacted] patients at a subcutaneous dose of 75 anti-Xa IU/kg/day for a period of ≤14 days or for treatment of deep vein thrombosis with and without pulmonary embolism at a subcutaneous dose of 175 anti-Xa IU/kg/day for at least 6 days. The sponsor has conducted sufficient preclinical toxicology studies. In 1-year subcutaneous toxicity studies with tinzaparin in rats and dogs, toxic effects were generally consistent with anticoagulant properties of the test article. For the 1-year subcutaneous toxicology study in rats, swelling and hemorrhages were observed at the injection sites. Internal examinations found pallor of internal organs consistent with blood loss. Bone ash (% of femur) was decreased for animals receiving tinzaparin at 25 mg/kg/day and animals receiving the comparator, heparin at 12.5 mg/kg/day. For the 1-year subcutaneous toxicology study in dogs, swelling and hemorrhage at injection sites were observed in all dogs receiving tinzaparin. The target organ of toxicity was the spleen. For tinzaparin-treated dogs, contraction of the sinuses in the spleen were observed, which was probably related to blood loss. From a preclinical standpoint, the application is recommended for approval.

The label is not according to 21 CFR, 201.57 (April 1, 1999), and changes in text as outlined in the review portion are needed.

RECOMMENDATION:

From a preclinical standpoint, the application is recommended for approval.

/S/

Timothy W. Robison, Ph.D.

2-14-2000
Date

cc:
Orig NDA 20,484
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Robison
HFD -345/Dr. Viswanathan

/S/ 2/22/00

R/D Init.: J. Choudary 12/17/99

TWR/hw/1/27/00, 2/1/00 & 2/14/00