A Randomized, Double-Blind Study to Assess Serum Transaminase Elevations and Antibody Formation Following Repeat Subcutaneous Dosing of LMWH, UFH or the Novel Anticoagulant M118 in Healthy Volunteer

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INTRODUCTION

M118 (adomiparin sodium) is a novel, rationally engineered low molecular weight heparin (LMWH) being developed as a baseline anticoagulant for use in the medical management of patients diagnosed with acute coronary syndrome (ACS). In a previous study evaluating M118 administered subcutaneously twice daily for 5 days, asymptomatic, reversible serum transaminase (AST and ALT) elevations were observed. Anti-heparin platelet (PLT) antibodies (Abs) were also detected by enzyme-linked immunosorbent assay (ELISA) in several subjects at Day 33 following M118 dosing. None of the subjects that developed Abs, as measured by ELISA assay, had a confirmed positive serotonin release assay (SRA).

Transaminase elevations have been reported in the literature following subcutaneous (s.c.) administration of unfractionated heparin (UFH) and LMWHs and are described in the package inserts of these products. An association between UFH and elevation in hepatic transaminases had been observed over 35 years ago. However, as these serum hepatic transaminase elevations have been asymptomatic, not usually systematically measured, and return to normal limits after therapy discontinuation, this phenomenon is not well recognized by clinicians. [1,2]

Enoxaparin sodium and dalteparin sodium are LMWHs with antithrombotic properties. Asymptomatic increases in AST and ALT greater than 3x upper limit of normal (ULN) have been reported in up to 6.1% and 5.9% of patients treated with enoxaparin, respectively. In clinical trials of dalteparin supporting non-cancer indications where hepatic transaminases were measured, asymptomatic increases in transaminase levels (AST and ALT) greater than 3x ULN were seen in 4.7% and 4.2% of patients during treatment, respectively.[3,4]

It is important for clinicians to recognize that administration of UFH and LMWH can cause transaminase elevation, and also to note that these elevations are described in package inserts as being fully reversible and rarely associated with increases in total bilirubin. Additionally, since aminotransferase determinations are important in the differential diagnosis of myocardial infarction, liver disease, and pulmonary emboli, elevations that might be caused by heparins and LMWH should be interpreted with caution.[3]

The present study was undertaken to gain more information on the elevation of hepatic transaminase levels and anti-heparin PLT Ab formation (as determined by ELISA) and function (as determined by SRA) within the same class of drugs, in a controlled comparative parallel group study in healthy male subjects.

Rationale for dose selection:

Enoxaparin, dalteparin: Since M118 s.c. injection is being developed for use in patients diagnosed with ACS, the dose from the package insert for ACS was selected for the comparative treatments (enoxaparin sodium, dalteparin sodium).

M118: The anticipated range of M118 doses for the indication of ACS is 100 – 150 anti-Xa IU/kg b.i.d.. A M118 dose of 125 anti-Xa IU/kg was shown in a previous study to elicit both reversible elevations of serum transaminase elevations and heparin/PF4 antibody formation.

UFH: A dose of 150 U/kg was selected for UFH to more closely approximate the level of anticoagulation observed with the doses of M118, enoxaparin and dalteparin used in this study.

OBJECTIVES

Primary Objectives

- To compare serum transaminase enzyme levels of UFH, enoxaparin sodium, dalteparin sodium and M118 (adomiparin sodium).
- To evaluate the occurrence of heparin/PF4 Ab formation and function.

Primary Endpoints

- The time-course and magnitude of increase above the ULN of hepatic transaminase enzymes levels (AST and ALT), alkaline phosphatase (ALP) and bilirubin.
- The occurrence of heparin/PF4 antibody as assessed by ELISA and SRA. Antibody titers and isotypes of positive subjects were assessed.

METHODOLOGY

Study Population

A total of 48 healthy male subjects between the ages of 18 and 50 years were enrolled. All subjects satisfied the inclusion and exclusion criteria, with verification at check-in. All subjects completed the clinical phase of the study and there were no subjects who were withdrawn/discontinued from the study.

Design

Subjects were randomized (12 subjects per dosing treatments) to receive b.i.d s.c. administrations of UFH, enoxaparin sodium, dalteparin sodium, or M118 for 4 days followed by a single dose on Day 5. All treatments were prepared on a weight-adjusted basis, per subject. Subjects were confined to the clinic from at least 15 hours prior to the first scheduled morning dose (Day -1) until 48 hours after the last dose (Day 7). Subjects returned for a follow-up visit 7 days after the last dose (Day 12), and for a final safety evaluation to test for the development of heparin-induced Abs 28 days after the last dose (Day 33).

Dosage

Treatment	Dose
A	150 U/kg UFH s.c.
B	1 mg/kg (100 IU/kg) enoxaparin sodium
C	120 IU/kg dalteparin sodium SC (maximum of 10,000 IU)
D	125 IU/kg M118 s.c.

Blood samples for safety assessment of liver function (AST, ALT, ALP, and bilirubin) were obtained from samples collected for serum chemistry before dosing on Day 1 (baseline), prior to each morning dose on Days 2 to 5, at 24 and 48 hours after the last dose (Day 6 and Day 7), and at the follow-up visit on Day 12.

Blood samples to assess the development of heparin/PF4 Abs were collected at screening (samples were analyzed by ELISA only) and on Days 7 and 12 (based on both ELISA and SRA). Subjects returned for a final safety evaluation 28 days after the last dose (Day 33) to assess the development of heparin/PF4 Abs through both the formation of heparin-induced PLT Abs (ELISA) and by an SRA. Subjects who developed heparin/PF4 Abs returned 60 days after the last dose and then at 30 day intervals, or until the Principal Investigator (PI) judged the level to be not clinically significant.

RESULTS

Figure 1: Mean ALT and AST Results

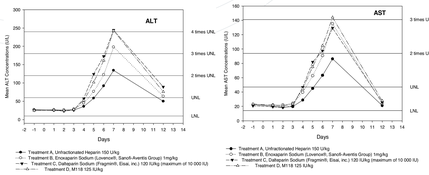


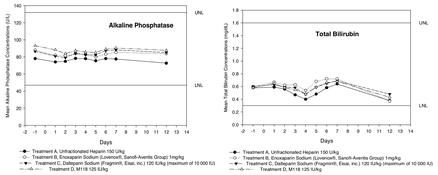
Table 1: WHO Toxicity Grades for ALT and AST Results by Treatment

Toxicity Grade	ALT (n = 48) Treatment				AST (n = 48) Treatment			
	UFH (A)	Enoxaparin (B)	Dalteparin (C)	M118 (D)	UFH (A)	Enoxaparin (B)	Dalteparin (C)	M118 (D)
Grade 0 (within normal limits)	2	0	0	1	3	0	1	1
Grade 1 (> ULN - 2.5 x UNL)	5	5	5	4	5	6	5	4
Grade 2 (> 2.5 - 5.0 x UNL)	4	4	4	3	4	4	4	4
Grade 3 (> 5.0 - 20.0 x UNL)	1	3	3	4	0	2	2	3
Grade 4 (> 20.0 x UNL)	0	0	0	0	0	0	0	0

Note: The highest grade was considered.

A Grade 3 toxicity occurred in 11 subjects (23%) with out-of-range ALT values and 7 subjects (15%) with out-of-range AST values; these were documented as AEs in the study database. Grade 2 toxicities occurred in 15 subjects (31%) for ALT and 16 subjects (33%) for AST values, respectively. Toxicity Grades of 0 or 1 were observed in 22 subjects (46%) for ALT and 25 subjects (52%) for AST. There were no Grade 4 toxicities.

Figure 2: Mean Alkaline Phosphatase and Total Bilirubin Results

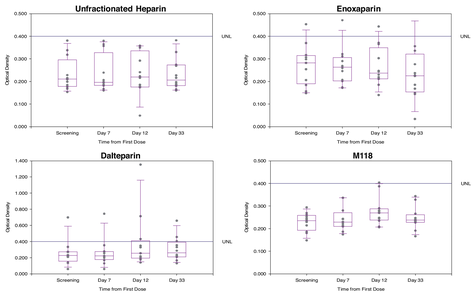


Antibody Titer and Isotype Response

EIA-GTI (combined IgG/M/A) assay was considered positive if the optical density was ≥ 0.4. EIA-IgG, EIA-IgM, and EIA-IgA subtype assays were considered positive if the optical density was ≥ 0.45. SRA was considered positive if the percentage of serotonin release was ≥ 20% which suggests the potential of developing (HIT) during clinical use.

Individual SRA values were within reference range (< 20%) at Days 7, 12, and 33 for all treatments.

Figure 3: Heparin-Induced PLT Ab (GTI-ELISA) Results by Treatment



The individual results and the median of heparin-induced PLT Abs results by treatment are presented in Figure 3 above.

Heparin-induced PLT Ab values greater than the reference range (≥ 0.4), with a negative SRA result (<20%), were reported for 3 subjects dosed with 120 IU/kg dalteparin sodium and for 1 subject dosed with 1 mg/kg enoxaparin sodium. Three (3) of the 4 subjects returned on Day 60 for follow-up testing. All follow-up results were still out-of-range (EIA-GTI-positive, weak IgM Abs, no IgG Abs, and SRA-negative); they were deemed not clinically significant by the PI. These results suggest that while there was a moderate Ab response specific for IgA and/or IgM, the response was not strong enough to activate platelets and generate a positive result in the SRA.

In total, 4 subjects (8%) were found to have either borderline or weak positive heparin-platelet Ab responses for at least one time point, as measured by ELISA as shown in Table 2. Two of these subjects had borderline positive values at baseline and remained positive through the 33 days study period. Two subjects developed weak positive antibodies over the dosing period.

Table 2: Heparin-Induced PLT Ab (GTI-ELISA) Positive Results (Optical Density Values)

I.D. Treatment	Days	Initial Value	Repeat	+High Heparin	IgG	Subtypes		
						IgG	IgM	IgA
GTI-EIA - negative baseline values								
20 Dalteparin	Baseline	0.140	0.376	0.176	0.166	0.136	0.277	0.165
	7	0.168	0.143	0.188	0.266	0.212	0.381	0.241
	12	0.431	0.440	0.170	0.213	0.171	0.570	0.287
	33	0.405	0.590	0.192	0.224	0.186	0.460	0.262
47 Dalteparin	Baseline	0.059	0.153	0.161	0.107	0.189	0.164	0.317
	7	0.059	0.194	0.206	0.187	0.209	0.271	0.513
	12	1.353	1.353	0.446	0.446	0.252	0.536	0.593
	33	0.456	0.801	0.306	0.283	0.185	0.357	0.634
GTI-EIA – positive baseline values								
30 Enoxaparin	Baseline	0.453	0.420	0.226	0.259	0.328	0.373	0.202
	7	0.471	0.549	0.245	0.393	0.412	0.646	0.26
	12	0.443	0.627	0.255	0.359	0.350	0.662	0.245
	33	0.516	0.721	0.217	0.363	0.410	0.866	0.318
38 Dalteparin	Baseline	0.698	0.435	0.220	0.211	0.373	0.405	0.209
	7	0.744	0.692	0.235	0.373	0.435	0.469	0.388
	12	0.713	0.613	0.302	0.435	0.422	0.730	0.408
	33	0.658	0.740	0.171	0.422	0.439	0.484	0.448

Table 3: Number (%) of Subjects With Adverse Events by System Organ Class

System Organ Class	Treatment				
	UFH (A) n= 12	Enoxaparin (B) n= 12	Dalteparin (C) n= 12	M118 (D) n= 12	**Total n= 48**
Gastrointestinal disorders	0 (0%)	1 (8%)	2 (17%)	1 (8%)	4 (8%)
General disorders and administration site conditions	11 (92%)	12 (100%)	11 (92%)	7 (58%)	41 (85%)
Investigations	1 (8%)	3 (25%)	3 (25%)	4 (33%)	11 (23%)
Musculoskeletal and connective tissue disorders	0 (0%)	1 (8%)	1 (8%)	1 (8%)	3 (6%)
Nervous system disorders	0 (0%)	0 (0%)	1 (8%)	1 (8%)	2 (4%)
Reproductive system and breast disorders	0 (0%)	0 (0%)	1 (8%)	0 (0%)	1 (2%)
Respiratory, thoracic and mediastinal disorders	2 (17%)	1 (8%)	0 (0%)	0 (0%)	3 (6%)

CONCLUSIONS

- Consistent with both literature reports and data reported from previous studies conducted by Momenta Pharmaceuticals, Inc. (MOM M118-102 and MOM M118-107), elevations in ALT and AST were observed, as expected, beginning at approximately 72 hours following initiation of dosing in all groups, peaking 24 - 48 hours post cessation of dosing, and generally returning to normal by 1 week post cessation of dosing.
- No clinical signs or symptoms of liver toxicity were observed and no elevations in bilirubin above the UNL were noted in any subject, at any time point. The incidence and magnitude of transaminase elevations were generally similar across all treatment groups. In particular, the M118 and dalteparin data were comparable.
- The data obtained in this comparative study are consistent with the known class effect of heparins and LMWH in causing transaminase elevations that do not appear to confer an increased risk of drug-induced liver injury.
- Anti-heparin/PF4 Ab generation was low in all groups. There were no positive results as determined by the SRA assay, considered to be the "gold standard" for differential diagnosis of heparin-induced thrombocytopenia (HIT).
- No meaningful conclusions can be drawn with respect to the likelihood of M118 to cause HIT relative to UFH and other LMWH preparations; however, there appears to be no obvious signal of increased risk of M118 relative to other heparin-based treatments evaluated in this study.
- Safety profiles of all heparin products utilized in this study were comparable as observed in Table 3.

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