Efficacy of M118, a Rationally-Engineered Low-Molecular-Weight Heparin, in a Canine Model of Arterial Thrombosis

Ian D Fier*, Mark A Nedelman, J Luis Guerrero*, Ganeesh Venkataraman* and Yi Wei Qi*

*Momenta Pharmaceuticals, Inc., Cambridge, MA; **Advanced Research Models, Nantong, China

ABSTRACT

Background: M118 is a novel, rationally-engineered, low-molecular-weight heparin (LMWH) under development for stable angina and acute coronary syndromes with favorable pharmacokinetics for subcutaneous or intravenous administration. In a canine model of arterial thrombosis, M118 significantly reduced clotting time and occlusion rate compared with unfractionated heparin (UFH).

Methods: Rats were randomly assigned to 24 groups in 4 experiments and followed a specific diet. A stable occlusion model was used to induce arterial thrombosis. Animals with untreated arterial thrombosis were treated with M118 administered subcutaneously or intravenously. Arterial occlusion rate, clotting time, platelet aggregation, and anti-Factor Xa activity were recorded.

Results: M118 significantly reduced arterial occlusion rate at all concentrations tested compared with untreated arterial thrombosis (Figure 1). The enhanced antithrombotic activity of M118 was not associated with increased cutaneous bleeding. Anti-Factor Xa activity observed in these experiments further distinguishes M118 from currently available LMWHs.

Conclusions: M118 has several key positive features of unfractionated heparin, including monitorability, reversibility, and simpler pharmacokinetics, which could simplify its use in patients undergoing PCI to treat stable angina and ACS.

INTRODUCTION

Rats were randomly assigned to 24 groups in 4 experiments and followed a specific diet. A stable occlusion model was used to induce arterial thrombosis. Animals with untreated arterial thrombosis were treated with M118 administered subcutaneously or intravenously. Arterial occlusion rate, clotting time, platelet aggregation, and anti-Factor Xa activity were recorded.

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Conclusions: M118 has several key positive features of unfractionated heparin, including monitorability, reversibility, and simpler pharmacokinetics, which could simplify its use in patients undergoing PCI to treat stable angina and ACS.

RESULTS

Figure 6. Correlation of anti-Factor Xa and IIa activities.

Figure 7. Anti-Factor Xa activity vs. time as assayed by ACT (left), aPTT (center), and PT assays (right).

Figure 8. Correlation of anti-Factor Xa and IIa activities.

Figure 9. Correlation of anti-Factor Xa and IIa activities.

Figure 10. Correlation of anti-Factor Xa and IIa activities.

REFERENCES


Figure 11. Summary of Selected Hematologic Endpoints

Table 1. Summary of Selected Hematologic Endpoints

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>UFH (75 U/kg)</th>
<th>M118 (75 lU/kg)</th>
<th>M118 (150 lU/kg)</th>
<th>M118 (37.5 lU/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Xa Activity</td>
<td>1.20 ± 0.16</td>
<td>1.06 ± 0.09</td>
<td>0.64 ± 0.12</td>
<td>1.15 ± 0.08</td>
</tr>
<tr>
<td>Anti-IIa Activity</td>
<td>8.0 ± 0.0</td>
<td>9.0 ± 0.0</td>
<td>8.0 ± 0.0</td>
<td>8.0 ± 0.0</td>
</tr>
</tbody>
</table>

ANIMALS AND REGISTERS

This study was carried out at Harlan Wistar Institute (Mississauga, ON, Canada) using 240 male Sprague-Dawley rats, 12 weeks of age. The rats were anesthetized with isoflurane, and arterial occlusion was induced by applying an electrical current (300 µA) to the left femoral artery for 180 minutes. Following occlusion of the left femoral artery, an IV catheter was inserted into the right femoral artery for monitoring continuous arterial blood pressure, blood flow, and heart rate. The rats were then injected with UFH at 75 U/kg + 1 U/kg/min, M118 at 75 lU/kg + 1 lU/kg/min, or a saline control (vehicle). Arterial occlusion was defined as a Doppler flow rate of 0.0 ± 0.0 mmHg or 0.0 ± 0.0 beats per minute, respectively, for 3 minutes or more. Statistical analysis was performed using Student’s t test assuming equal variance in different treatment groups. The primary endpoint was the time to arterial occlusion. The secondary endpoints were clotting time and arterial thrombosis rate.

RESULTS

Figure 5. Effect of M118 treatment on clotting time and occlusion rate.

Figure 6. Correlation of anti-Factor Xa and IIa activities.

Figure 7. Anti-Factor Xa activity vs. time as assayed by ACT (left), aPTT (center), and PT assays (right).

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ABSTRACT
INTRODUCTION
OBJECTIVE
Heparin derivatives, including unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH), play a central role in treatment paradigms for acute coronary syndromes (ACS), risk, in a canine model of deep arterial thrombosis induced by severe electrolytic injury.

Methods: M118 was supplied by Momenta Pharmaceuticals, Inc. (Cambridge, MA, USA) and reconstituted in 0.9% sterile saline (UFH, 75 U/kg), and 0.9% saline (vehicle control). Both classes of agents have potent antithrombotic effects, but inactivation of Factor IIa, limited reversibility with protamine sulfate, and decreased monitorability by point-of-care assays (ie, activated clotting time [ACT]) are issues with UFH. M118, a novel LMWH with potent anti-Factor Xa and IIa activity, monitorability, and reversibility, offers several key advantages over UFH.

Results: Both M118 (75 and 37.5 lU/kg) and UFH (75 U/kg) were well tolerated and effective in reducing clotting time and inactivation of Factor IIa. M118 dose-dependent inhibition of clotting was observed in all coagulation assays (AT-III) and the linear heparin chain leads to capture and inactivation of thrombin (Factor IIa) and Factor Xa.

Conclusions: The study design, shown in Figure 1,

Figure 1: Study Design

- M118 (150 IU/kg)†
- M118 37.5 lU/kg + 1 IU/kg/min
- UFH (75 U/kg)
- 0.9% saline infusion n = 24

Figure 2: Anti-Factor IIa Activity (IU/mL) vs. Time After Current Initiation (min)

- M118 (150 IU/kg)
- M118 (75 IU/kg)
- UFH (75 U/kg)
- 0.9% saline infusion

Figure 3: Percentage of animals with fully occluded femoral arteries.

- M118 37.5 lU/kg + 1 IU/kg/min
- UFH (75 U/kg)
- 0.9% saline infusion

Figure 4: Anti-Factor Xa Activity (IU/mL) vs. Time After Current Initiation (min)

- M118 (150 IU/kg)
- M118 (75 IU/kg)
- UFH (75 U/kg)
- 0.9% saline infusion

Figure 5: Clotting Time (sec) vs. Time After Current Initiation (min)

- M118 (150 IU/kg)
- M118 (75 IU/kg)
- UFH (75 U/kg)
- 0.9% saline infusion

Figure 6: Results

- M118 37.5 lU/kg + 1 IU/kg/min
- UFH (75 U/kg)
- 0.9% saline infusion

Table 1: Summary of Results

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Anti-Factor IIa (IU±SD)</th>
<th>Anti-Factor Xa Activity (IU±SD)</th>
<th>Clotting Time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFH (75 U/kg)</td>
<td>&lt;0.05 vs. M118 [150 IU/kg], despite comparable values</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M118 (75 IU/kg)</td>
<td>⤣</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M118 (37.5 IU/kg) + 1 IU/kg/min</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Values were reported as means ± standard error (SE) unless otherwise noted. Means were compared using the non-parametric Mann-Whitney U-test. Error bars are ± SE. UFH, unfractionated heparin.

*Recorded at 60 min after initiation of test article infusion; †Bolus dose (see Figure 1).
**ABSTRACT**

**Background:** M118 is a novel, rationally-engineered, low-molecular-weight heparin (LMWH) under development for stable angina and acute coronary syndromes with favorable pharmacokinetics for subcutaneous or intravenous administration, potent anti-Factor Xa and IIa activity, constant anti-Factor Xa:IIa ratio over time, rapid reversibility, and monitorability using standard point-of-care assays.

**Methods:** Right and left femoral arteries of 24 beagle dogs were instrumented with intravascular electrodes and perivascular Doppler flow probes. Fifteen minutes after initiating continuous saline infusion, thrombogenic injury was induced in the right (control) artery by applying an electrical current (300 µA) to the intima that was maintained continuously for 180 minutes. Following occlusion of the control artery, an IV bolus plus 90 minutes continuous infusion of M118 (37.5, 75 or 150 anti-Xa IU/kg + 1 anti-Xa IU/kg/min, n=6/dose) or unfractionated heparin (UFH; 75 U/kg + 1 U/kg/min, n=6) was administered, and the left (active) artery was injured in a similar manner.

**Results:** Incidence of occlusion was higher in arteries treated with saline (24/24) or UFH (5/6) than with M118 (3/6 low; 2/6 middle; 1/6 high dose, P<0.05, UFH vs. M118 [150 IU/kg]), despite comparable anticoagulation levels (ACT, PT, aPTT) between UFH and M118 (150 anti-Xa IU/kg). Time to occlusion was significantly prolonged by UFH and M118 at all doses compared to saline (P<0.01), but only minimal increases in cutaneous bleeding time were observed in all M118 and UFH treatment groups. M118 exhibited consistent anti-Factor Xa:IIa ratios over time at all doses.

**Conclusions:** M118, a novel LMWH with potent anti-Factor Xa and IIa activity, monitorability, and reversibility, demonstrated superior efficacy at 150 anti-Xa IU/kg to a standard dose of UFH, without increased bleeding risk, in a canine model of deep arterial thrombosis induced by severe electrolytic injury.

**INTRODUCTION**

- Heparin derivatives, including unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH), play a central role in treatment paradigms for acute coronary syndromes (ACS), particularly as adjunctive therapy during PCI.

- Both classes of agents have potent antithrombotic effects, but also limitations. UFH has unpredictable patient-dependent pharmacokinetics and the potential to induce platelet aggregation and heparin-induced thrombocytopenia. LMWH has reduced inhibition of Factor IIa, limited reversibility with protamine sulfate, and decreased monitorability by point-of-care assays (ie, activated clotting time [ACT] assays).

- M118 (Figure 1) is a novel rationally-engineered LMWH under development for the treatment of stable angina and ACS with favorable pharmacokinetics for subcutaneous or intravenous administration, potent anti-Factor Xa and IIa activity, constant anti-Factor Xa:IIa ratio over time, rapid reversibility, and monitorability using standard point-of-care assays.

**OBJECTIVE**

- To examine the antithrombotic and anticoagulant effects of M118 in a canine model of acute arterial thrombosis.

**METHODS**

**ANIMALS AND REAGENTS**

- This study was carried out at Charles River Laboratories (Worcester, MA, USA) using 24 purpose-bred, male, beagle dogs supplied by Marshall BioResources (North Rose, NY, USA).

- Husbandry conditions and surgical procedures were managed in accordance with the Guide for the Care and Use of Laboratory Animals. Euthanasia was conducted in compliance with accepted American Veterinary Medical Association guidelines.

- M118 was supplied by Momenta Pharmaceuticals, Inc. (Cambridge, MA, USA) and reconstituted in 0.9% sterile saline. Unfractionated heparin (heparin sodium injection, USP; 5000 U/mL in 0.9% sterile saline) was obtained from Baxter Healthcare Corporation (Deerfield, IL, USA).

**ANIMAL INSTRUMENTATION**

- Intravascular electrodes were inserted through the left and right femoral artery walls, positioned in direct contact with the intima, and connected to a constant amperage power source.

**Figure 1. M118 interactions with coagulation proteins.**

M118-induced conformational changes in antithrombin (AT-III) and the linear heparin chain leads to capture and inactivation of thrombin (Factor IIa) and Factor Xa.
- A stenotic device and a perivascular Doppler flow probe were positioned immediately distal and proximal, respectively, to the intravascular electrode.
- Catheters were inserted into the carotid artery for monitoring continuous arterial blood pressure, blood flow, and heart rate and into the jugular vein for blood sample collection.
- An intravenous line was inserted into a peripheral vein for study treatment infusions.

**ELECTROLYTIC–INJURY-INDUCED THROMBOSIS**

- The study design, shown in Figure 2, was based on the model system developed by Lucchesi and colleagues.4,4
- For each artery, the distal stenotic device was adjusted to limit reactive hyperemia to ≤80% of the baseline response to physical occlusion. In addition, mean arterial pressure and heart rate were maintained at approximately 70 mm Hg and 100 beats per minute, respectively, via isoflurane anesthetic management.
- Total occlusion was defined as a Doppler flow ≤2% of baseline for at least 1 minute without cycling.

**RESULTS**

- Activated clotting time (ACT), prothrombin time (PT), activated partial thromboplastin time (aPTT), anti-Factor Xa and IIa levels, and cutaneous bleeding time (CBT) were determined by standard methodologies at 15 minutes, 60 minutes, and either 180 minutes or the time of full occlusion, if earlier.

**HEMATOLOGY AND COAGULATION PARAMETERS**

- Values were reported as means ± standard error (SE) unless otherwise noted. Means were compared using the Student's t test assuming equal variance in different treatment groups.
- The incidence of occlusion was compared between treatment groups by calculating the odds ratio relative to control and using a z test to derive the P-value.

**Figure 2. Study design.**

**Figure 3. Percentage of animals with fully occluded femoral arteries.**

Animals were monitored by Doppler flow for up to 180 minutes post current initiation. Full occlusion was defined as a reduction in Doppler flow to ≤2% of baseline values for at least 1 minute without cycling. UFH, unfractionated heparin.

* P<0.05 vs. control; † P<0.02 vs. UFH.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animals with Occluded Arteries (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>(24/24)</td>
</tr>
<tr>
<td>UFH (75 U/kg)</td>
<td>(5/6)</td>
</tr>
<tr>
<td>M118 (37.5 IU/kg)</td>
<td>(3/6)</td>
</tr>
<tr>
<td>M118 (75 IU/kg)</td>
<td>(2/6)</td>
</tr>
<tr>
<td>M118 (150 IU/kg)</td>
<td>(1/6)</td>
</tr>
</tbody>
</table>

**Table 1. Summary of Selected Hematologic Endpoints**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Clotting Time (sec)</th>
<th>Anti-Factor IIa Activity (lU/mL)</th>
<th>Anti-Factor Xa Activity (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>163 ± 55</td>
<td>0.8 ± 0.1</td>
<td>2.89 ± 1.31</td>
</tr>
<tr>
<td>UFH (75 U/kg)</td>
<td>160 ± 55</td>
<td>1.0 ± 0.1</td>
<td>2.59 ± 1.22</td>
</tr>
<tr>
<td>M118 (75 IU/kg)</td>
<td>108 ± 13</td>
<td>0.6 ± 0.1</td>
<td>1.66 ± 0.37</td>
</tr>
<tr>
<td>M118 (37.5 IU/kg)</td>
<td>101 ± 7</td>
<td>0.5 ± 0.1</td>
<td>1.20 ± 0.16</td>
</tr>
</tbody>
</table>

**Figure 5. Anti-Factor Xa activity (IU/mL)**

**Figure 6. Correlation of anti-Factor Xa and IIa activities.**
M118, a novel LMWH with potent anti-Factor Xa and IIa activity, monitorability, and reversibility, had significantly greater antithrombotic efficacy at all tested concentrations than vehicle. M118 at 150 IU/kg had significantly greater antithrombotic efficacy than UFH at 75 U/kg. The ratio of anti-Factor Xa activity to IIa activity over time was generally more constant with M118 than UFH. The enhanced antithrombotic activity of M118 was not associated with increased cutaneous bleeding. The incidence of occlusion was compared between treatment groups by calculating the odds ratio relative to baseline and using a z test to derive the P value.

The ratio of anti-Factor Xa activity to IIa activity over time was generally more constant with M118 than UFH. The enhanced antithrombotic activity of M118 was not associated with increased cutaneous bleeding. The incidence of occlusion was compared between treatment groups by calculating the odds ratio relative to baseline and using a z test to derive the P value.

The vehicle control group shown in the graphs subsequently received M118 at 150 IU/kg. Error bars are ± SE. UFH, unfractionated heparin. *aPTT values >212 seconds not included.

The vehicle control group shown in the graphs subsequently received M118 at 150 IU/kg. Error bars are ± SE. UFH, unfractionated heparin.

The vehicle control group shown in the graphs subsequently received M118 at 150 IU/kg. Error bars are ± SE. UFH, unfractionated heparin.

Individual points represent data from a single animal. All animals in all treatment groups are shown. Correlation coefficients (r²) were 0.890 and 0.465 in the M118 and unfractionated heparin (UFH) groups, respectively.

**Table 1. Summary of Selected Hematologic Endpoints**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ACT (sec)*</th>
<th>Anti-Factor Xa (IU±SD)*</th>
<th>Anti-Factor IIa (IU±SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>64 ± 18</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>M118 (37.5 IU/kg)</td>
<td>101 ± 7†</td>
<td>1.20 ± 0.16</td>
<td>0.64 ± 0.12</td>
</tr>
<tr>
<td>M118 (75 IU/kg)</td>
<td>108 ± 13‡</td>
<td>1.66 ± 0.37</td>
<td>0.71 ± 0.20</td>
</tr>
<tr>
<td>M118 (150 IU/kg)</td>
<td>141 ± 28</td>
<td>2.31 ± 0.13‡</td>
<td>1.06 ± 0.09‡</td>
</tr>
<tr>
<td>UFH (75 IU/kg)</td>
<td>163 ± 55‡</td>
<td>2.89 ± 1.31</td>
<td>0.95 ± 0.19</td>
</tr>
</tbody>
</table>

ACT, activated clotting time; IU, international unit; SD, standard deviation, UFH, unfractionated heparin.

*Recorded at 60 min after initiation of test article infusion; †Bolus dose (see Figure 2); ‡P<0.05, M118 vs. UFH; †P<0.01, UFH vs. control.

**REFERENCES**


M118, a novel LMWH with potent anti-Factor Xa and IIa activity, monitorability, and reversibility, had significantly greater antithrombotic efficacy at all tested concentrations than vehicle (Figure 3).

M118 at 150 IU/kg had significantly greater antithrombotic efficacy than UFH at 75 U/kg (Figure 3). The enhanced antithrombotic activity of M118 was not associated with increased cutaneous bleeding time relative to the UFH group (data not shown).

M118 dose-dependent inhibition of clotting was observed in all coagulation assays (Figure 4 and Table 1). Unlike other LMWHs, M118 may therefore have the potential to be assayed by the point-of-care ACT assay, which could simplify its use in patients undergoing PCI to treat stable angina and ACS.

M118 exhibited dose-dependent inhibition of both Factor Xa and IIa (Figure 5 and Table 1). Anti-Factor IIa activity increased linearly with anti-Factor Xa activity for both M118 and UFH, although the correlation coefficient was greater for M118 (r²=0.890) than UFH (r²=0.465) (Figure 6). The prominent anti-Factor IIa activity observed in these experiments further distinguishes M118 from currently available LMWHs.

The ratio of anti-Factor Xa activity to IIa activity over time was generally more constant with M118 than UFH, consistent with the known variable metabolism of the large and polydisperse UFH molecules (Figure 7).

By combining potent anti-Factor Xa and IIa activity, consistent antithrombotic effects over time, easy monitorability, reversibility, and simpler pharmacokinetics, M118 has several key positive features of both LMWH and UFH and, therefore, may be an attractive option in the future for the treatment of stable angina and ACS.

REFERENCES