PHARMACOKINETICS WRITTEN SUMMARY

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## ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>One-way analysis of variance</td>
</tr>
<tr>
<td>β-TCP</td>
<td>Beta-tricalcium phosphate</td>
</tr>
<tr>
<td>CNAA</td>
<td>Complete new attachment apparatus</td>
</tr>
<tr>
<td>DFDBA</td>
<td>Demineralised freeze-dried bone allograft</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>HDPE</td>
<td>High density polyethylene</td>
</tr>
<tr>
<td>INN</td>
<td>International non-proprietary name</td>
</tr>
<tr>
<td>MEM</td>
<td>Minimum essential medium</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet-derived growth factor</td>
</tr>
<tr>
<td>PETG</td>
<td>Glycol-modified polyethylene terephthalate</td>
</tr>
<tr>
<td>PII</td>
<td>Primary irritation index</td>
</tr>
<tr>
<td>PMA</td>
<td>Premarket Approval Application</td>
</tr>
<tr>
<td>rhPDGF-BB</td>
<td>Recombinant human platelet-derived growth factor BB homodimer</td>
</tr>
<tr>
<td>SBA</td>
<td>Summary basis of approval (FDA)</td>
</tr>
<tr>
<td>U.S. FDA</td>
<td>United States Food and Drug Administration</td>
</tr>
</tbody>
</table>
2.6 NONCLINICAL SUMMARY
2.6.4 Pharmacokinetics Written Summary
2.6.4.1 Brief Summary

A critical aspect for the use of Augment treatment to enhance fracture repair is the release characteristics of rhPDGF-BB from the matrix with which it is combined. BioMimetic has conducted a series of in vivo and in vitro studies assessing the release characteristics of $^{125}$I-labeled rhPDGF-BB from multiple types of matrices, all of which contain $\beta$-TCP as an osteoconductive scaffold. Study reports are provided in Appendix 2.6.4.1-1.

Two in vivo studies assessed the release of $^{125}$I-rhPDGF-BB when implanted in a rat calvarial bone defect. This model was selected to mimic the placement of rhPDGF-BB at the site of a bone defect containing bleeding bone, as occurs with the use of Augment for the treatment of foot and ankle fusions. Additionally, a series of in vitro studies were conducted to demonstrate that combining the rhPDGF-BB with $\beta$-TCP has no impact on the chemical stability or biological activity of the protein. The methods used to assess chemical and biological stability are procedures that are routinely used as part of our ongoing program to monitor stability of formulated rhPDGF-BB.

2.6.4.2 Methods of Analysis
2.6.4.2.1 Release of $^{125}$I-rhPDGF-BB from $\beta$-TCP Containing Matrices Implanted in a Rat Calvarial Bone Defect

The purpose of this study was to evaluate the in vivo release of $^{125}$I-rhPDGF-BB from two different $\beta$-TCP matrix materials after implantation in calvarial defects in rats. The two matrix materials consisted of: (1) $\beta$-TCP with nominal particle size of 250 - 1000 $\mu$m and (2) $\beta$-TCP with nominal particle size of 100 - 300 $\mu$m combined with a collagen binder.

Study Procedure

Eight (8) Sprague-Dawley rats were used in this study. They were randomised between the two (2) groups to receive $^{125}$I-rhPDGF-BB combined with either $\beta$-TCP/collagen matrix (Group I) or $\beta$-TCP alone (Group II). The $^{125}$I-rhPDGF-BB was first added to unlabelled rhPDGF-BB that was at 0.3 mg/mL to provide a total rhPDGF-BB concentration that would mimic the intended clinical concentration of rhPDGF-BB being evaluated in foot and ankle
fusion procedures. Approximately $1.0 \times 10^7$ cpm of $^{125}$I-rhPDGF-BB was used per animal. The $^{125}$I-rhPDGF-BB preparation was added to the appropriate matrix and 0.1 mL of hydrated matrix then applied to an 8 mm diameter, critical-sized calvarial defect in each of the four (4) rats of each of the two (2) groups. Measurements of radioactive counts at calvarial defect sites were performed using a hand-held Geiger counter at an optimal fixed distance of 1 inch above the defect site. Counts were taken immediately after implantation, then at 5, 15, 30, 60 minutes, 2, 4, 8, 24, 48, 72 hours and at 7 days after implantation. The number of radioactive counts measured at each time was compared to the time zero counts and the results plotted against time to illustrate the release of $^{125}$I-rhPDGF-BB \textit{in vivo} from the test matrices at each defect site. The test articles are further described in Table 2.6.4-1.

<table>
<thead>
<tr>
<th>Test Article</th>
<th>Supplier</th>
<th>Physical Description</th>
<th>Storage Conditions</th>
<th>Lot Number</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-TCP/Collagen</td>
<td>Kensey Nash</td>
<td>White sponge</td>
<td>Room temperature</td>
<td>187-03</td>
<td>N/A</td>
</tr>
<tr>
<td>(80%/20%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-TCP (250 - 1000 µm)</td>
<td>BioMimetic Therapeutics</td>
<td>White, granular powder</td>
<td>Room temperature</td>
<td>BN0091A</td>
<td>N/A</td>
</tr>
<tr>
<td>rhPDGF-BB</td>
<td>BioMimetic Therapeutics</td>
<td>Liquid</td>
<td>-70°C</td>
<td>AAI-022006-3</td>
<td>1.0 mg/mL</td>
</tr>
<tr>
<td>$^{125}$I-rhPDGF-BB</td>
<td>GE Healthcare/Amersham</td>
<td>Liquid</td>
<td>4°C</td>
<td>IMQ.7721</td>
<td>333 µCi/mL</td>
</tr>
</tbody>
</table>

All surgeries were performed under strict asepsis and in a room separate from where the radioactive material resided. A drill was used to carve out the margin of the defect (approximately 8 mm in diameter) in the parietal bone until the central piece of bone was completely free from attachment. After irrigation and bone removal, the defect was covered with an overlay of 0.1cc of the designated test article that had been spiked with $^{125}$I-rhPDGF-BB. A Geiger counter reading was immediately taken and constituted baseline (time = 0). The remaining measurement procedures (5 minutes through 7 days after implantation) were conducted with the animal in a non-anaesthetised state. Changes in counts measured at the implant site over time were used to determine the release rate of the radiolabelled rhPDGF-BB, calculating the percent of the initial counts remaining at each of...
the designated measurement times. The percent of counts present at the implant site were plotted against time to generate the in vivo $^{125}$I-rhPDGF-BB release profiles for both β-TCP/collagen and β-TCP.

**Results**

Radiolabelled $^{125}$I-rhPDGF-BB was released from β-TCP/collagen and β-TCP matrices in a similar manner in vivo (Figure 2.6.4-1; Table 2.6.4-2). During the initial minutes there was a rapid release of $^{125}$I-rhPDGF-BB measured at the surgical site for the β-TCP/collagen and β-TCP matrices, with approximately 80% and 70%, respectively, released in the first 60 minutes. After this time point there was a gradual decrease in $^{125}$I-rhPDGF-BB measured at the surgical site over the following 7 days for both matrix materials, with only 2% of the input counts present at the 7-day time point.

![Image](image-url)

**Figure 2.6.4-1:** Mean whole blood concentration values for Sprague-Dawley Rats Following Implantation of $^{125}$I- rhPDGF-BB Combined with β-TCP and β-TCP/collagen.

<table>
<thead>
<tr>
<th>Group</th>
<th>$^{125}$I-rhPDGF-BB - Mean % CPM Remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>I (n = 4)</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 2.6.4-2: Rat calvarial model - 125I-rhPDGF-BB release kinetics - study results

<table>
<thead>
<tr>
<th>II (n = 4)</th>
<th>100</th>
<th>85</th>
<th>73</th>
<th>41</th>
<th>31</th>
<th>27</th>
<th>25</th>
<th>19</th>
<th>7</th>
<th>8</th>
<th>8</th>
<th>2</th>
</tr>
</thead>
</table>

Group I: β-TCP/collagen matrix  
Group II: β-TCP

Conclusions

The results of this in vivo study demonstrated that, when placed in contact with live tissues in a rat calvarial defect, both β-TCP/collagen and β-TCP alone show similar release characteristics with a rapid delivery of rhPDGF-BB in the first 60 minutes, followed by a slow sustained release over 7 days. The results reported in this study for Group II (β-TCP matrix; 250 - 1000 µm) are consistent with the findings reported in Section 5.3.2 in which the same β-TCP matrix was analysed in a separate study (Group I in that study) designed to monitor in vivo release kinetics of 125I-rhPDGF-BB. In the two studies the mean percent of 125I-rhPDGF-BB remaining at 1 hour was 69 and 64%, and at 24 hours 93 and 86%.

2.6.4.2.2 Release of 125I-rhPDGF-BB from Various Matrices Implanted in a Rat Calvarial Bone Defect

A study was conducted to assess the release kinetics of 125I-rhPDGF-BB when implanted into a rat calvarial bone defect. The study was designed to evaluate three (3) test matrix materials for the in vivo release profile of 125I-rhPDGF-BB. The three (3) test materials included: Group I) β-TCP matrix with a 250 - 1000 µm particle size; Group II) β-TCP matrix with a 1000 - 2000 µm particle size (Augment); and Group III) 80%/20% (v/v) combination of freeze-dried human bone allograft (FDBA) and β-TCP with a 250 - 1000 µm particle size. This study was a non-GLP study, though all portions of the study were conducted in compliance with approved SOPs and/or in accordance with detailed study-specific procedures maintained in the study file.

Study Procedure

Approximately 1.0 x 10^7 cpm of 125I-rhPDGF-BB was used per animal. The test articles were applied to an 8 mm diameter, critical-sized calvarial defect in each of six (6) rats in each of the three (3) test groups. Measurements of radioactive counts at calvarial defect sites were performed using a Geiger counter at an optimal fixed distance of 1 inch above the defect site. Counts were taken immediately after implantation, than at 30 and 60 minutes, 2, 4, 6, 8, 24,
48, and 72 hours after implantation. The number of radioactive counts measured at each time was compared to the time zero counts and the results plotted against time to illustrate the release of $^{125}$I-rhPDGF-BB \textit{in vivo} from the test matrices at each defect site. The test articles are further described in Table 2.6.4-3.

<table>
<thead>
<tr>
<th>Test Article</th>
<th>Supplier</th>
<th>Physical Description</th>
<th>Storage Conditions</th>
<th>Lot Number</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-TCP (250-1000 µm)</td>
<td>BioMimetic Therapeutics</td>
<td>White, granular powder</td>
<td>Room temperature</td>
<td>BN0091A</td>
<td>N/A</td>
</tr>
<tr>
<td>β-TCP (1000-2000 µm)</td>
<td>BioMimetic Therapeutics</td>
<td>White, granular powder</td>
<td>Room temperature</td>
<td>BN0355A</td>
<td>N/A</td>
</tr>
<tr>
<td>Freeze-Dried Bone Allograft (FDBA)/β-TCP (250-1000 µm)</td>
<td>BioMimetic Therapeutics (FDBA supplied by LifeNet)</td>
<td>80%/20% vol/vol White, granular powder</td>
<td>Room temperature</td>
<td>03-0345 and BN0091A</td>
<td>N/A</td>
</tr>
<tr>
<td>rhPDGF-BB</td>
<td>BioMimetic Therapeutics</td>
<td>Liquid</td>
<td>2-8ºC</td>
<td>VA5105</td>
<td>10 mg/mL</td>
</tr>
<tr>
<td>$^{125}$I-rhPDGF-BB</td>
<td>GE Healthcare/Amersham</td>
<td>Liquid</td>
<td>2-8ºC</td>
<td>IMQ.7721 vBatch 0719</td>
<td>150 µCi/mL (shipment 1) 100 µCi/mL (shipment 2)</td>
</tr>
</tbody>
</table>

Eighteen (18) Sprague-Dawley rats were randomized into three groups prior to surgery. All surgeries were performed under strict asepsis and in a room separate from where the radioactive material resided. A drill was used to carve out the margin of the defect (approximately 8 mm in diameter) in the parietal bone until the central piece of bone was completely free from attachment. After irrigation and then removal, the defect was covered with an overlay of 0.1 cc of the designated test article spiked with $^{125}$I-rhPDGF-BB. A Geiger counter reading was immediately taken and constituted baseline (time = 0). The remaining measurement procedures (30 minutes through 72 hours after implantation) were conducted with the animal in a non-anaesthetized state. Changes in counts measured at the implant site over time were used to determine the release rate of the radiolabelled rhPDGF-BB, calculating the percent of the initial counts remaining at each of the designated measurement
times. The percent of counts present at the implant site were plotted against time to generate the *in vivo* $^{125}$I-rhPDGF-BB release profiles for the three matrix materials.

**Results**

$^{125}$I-rhPDGF-BB was released from $\beta$-TCP (250 - 1000 µm), $\beta$-TCP (1000 - 2000 µm), and FDBA/$\beta$-TCP matrices in a similar manner *in vivo*. During the initial 30 minutes there was a rapid loss of $^{125}$I-rhPDGF-BB measured at the surgical site of approximately 50% for all three test materials (Table 2.6.4-4; Figure 2.6.4-2). After this time point, there was a gradual decrease in $^{125}$I-rhPDGF-BB measured at the surgical site over the following 72 hours, with approximately 10% of the $^{125}$I-rhPDGF-BB counts remaining for all three groups at the final time point. There were no statistically significant differences between groups using the one-way ANOVA statistical analysis method.

<table>
<thead>
<tr>
<th>Group</th>
<th>0 min</th>
<th>30 min</th>
<th>1 hrs</th>
<th>2 hrs</th>
<th>4 hrs</th>
<th>8 hrs</th>
<th>24 hrs</th>
<th>48 hrs</th>
<th>72 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (n = 6)</td>
<td>100</td>
<td>49</td>
<td>36</td>
<td>29</td>
<td>25</td>
<td>21</td>
<td>14</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>II (n = 6)</td>
<td>100</td>
<td>45</td>
<td>37</td>
<td>34</td>
<td>35</td>
<td>25</td>
<td>21</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>III (n = 6)</td>
<td>100</td>
<td>47</td>
<td>45</td>
<td>44</td>
<td>34</td>
<td>28</td>
<td>20</td>
<td>15</td>
<td>13</td>
</tr>
</tbody>
</table>

*p* value: 1.00 0.839 0.311 0.078 0.298 0.182 0.183 0.121 0.187

**Table 2.6.4-4:** Rat calvarial model $^{125}$I-rhPDGF-BB release kinetics - study results

- **Group I:** 0.3 mg/mL rhPDGF-BB + $\beta$-TCP (250 - 1000 µm)
- **Group II:** 0.3 mg/mL rhPDGF-BB + $\beta$-TCP (1000 - 2000 µm)
- **Group III:** 0.3 mg/mL rhPDGF-BB + Allograft/$\beta$-TCP (250 - 1000 µm)
Figure 2.6.4-2: Mean whole blood concentration values for Sprague-Dawley Rats Following Implantation of $^{125}$I- rhPDGF-BB Combined with $\beta$-TCP and $\beta$-TCP/collagen.

Conclusions

The results of this in vivo study demonstrated that when placed in contact with live tissues in a rat calvarial defect, the $\beta$-TCP (250 - 1000 µm), $\beta$-TCP (1000 - 2000 µm), and FDBA/$\beta$-TCP matrices showed similar release characteristics with rapid release of the $^{125}$I-rhPDGF-BB in the first 30 minutes, followed by a slow sustained release over the following 72 hours.

2.6.4.3 Other Pharmacokinetic Studies

2.6.4.3.1 ADME - Tissue Distribution and Mass Balance of Radioactivity in Sprague-Dawley Rats Following an Intravenous Injection of $^{125}$I-rhPDGF-BB

Augment was developed for single local administration at the site of foot-and-ankle bone fusions. As a result of the treatment plan, significant systemic exposure is unlikely. In addition, once rhPDGF-BB is in the circulatory system it is rapidly cleared through the kidney and liver, with a short circulating half-life as demonstrated in the ADME study described in this section.

This study was conducted to evaluate the distribution, excretion and mass balance of $^{125}$I-rhPDGF-BB administered as a single intravenous (IV) dose to male CD® [Crl:CD®(SD)] Sprague-Dawley rats. Intravenous administration was chosen to provide safety data in an instance of a “worst-case scenario” of direct introduction of rhPDGF-BB into systemic circulation.

This nonclinical laboratory study was conducted in accordance with GLP regulations.

Study Procedure

One treatment group of four (4) males (Group 1) and one treatment group of fourteen (14) males (Group 2) were administered the test article via bolus IV injection at a total average dose level of 0.31 mg/kg with 22.30 µCi/animal (99.55 µCi/kg). The target volume dose for each group was 1 mL/kg.
Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. Body weights were measured and recorded on Day 1 prior to test article administration. Blood samples were collected at designated time points for whole blood radioactivity analysis. Urine, faeces, and cage residue were collected from Group 1 animals at designated intervals up to 168 hours post-dose for radioactivity concentration analysis. At designated intravenous dosing, the Group 2 animals were prepared and shipped out for quantitative whole body autoradiography (QWBA) analysis. At 168 hours post-dose, Group 1 animals were euthanized and the carcass was collected and analyzed for residual radioactivity concentration analysis.

Results

Adverse effects: No adverse effects were noted during the course of this study.

Whole blood concentrations of radioactivity: Whole blood concentration values are illustrated in Figure 2.6.4-3. Whole blood radioactivity was measured at a nominal sample time of 168 hours post-injection for the animals of Group 1 and at the time of euthanasia 30 minutes, and 1, 2, 4, 8, 24, 72, 120, and 168 hours after the intravenous injection for the animals of Group 2.

Figure 2.6.4-3: Mean whole blood concentration values for Group 2 - Sprague-Dawley Rats Following an IV injection of $^{125}$I-rhPDGF-BB.
Recovery of Radioactivity in Excreta, Cage Residue, and Carcass: Mean cumulative percent recoveries of radioactivity as a percentage of the administered dose in urine, faeces and cage residue are summarised in Figure 2.6.4-4.

![Cumulative recovery graph](image)

Figure 2.6.4-4: Mean Cumulative Percent Recovery of Radioactivity for Group 1 Animals Following IV injection of $^{125}$I-rhPDGF-BB.

Following intravenous administration of $^{125}$I-rhPDGF-BB, cumulative recoveries of the administered radioactivity averaged $107.89 \pm 0.87\%$ of the dose over the 168 hour study period. Of this, approximately 97% of the dose was recovered in the first 24 hours of the study. Renal excretion of the radioactivity averaged $68.54 \pm 8.56\%$. An additional $28.04 \pm 5.17\%$ of the dose was recovered in faeces and $8.64 \pm 2.84\%$ was recovered in cage rinse.

Approximately $2.65 \pm 1.34\%$ of the dose remained in the carcass at termination of the study 168 hours after the dose. Thus, a majority of the radioactivity associated with the test article was excreted in the urine. Based on the results of trichloroacetic acid (TCA) precipitation analyses of the urine samples most of the excreted radioactivity was unbound (Figure 2.6.4-5). The radioactivity excreted in faeces, in contrast to the urine samples, appeared to be partially bound. Approximately one third of radioactivity excreted in faeces was precipitated by the TCA procedure.
Tissue distribution of $^{125}$I: Drug-derived radioactivity was widely distributed to the tissues of male Sprague-Dawley rats after a single IV dose of $^{125}$I-rhPDGF-BB, as measured by QWBA. Tissues with the highest concentrations of radioactivity were thyroid, liver, small intestine, kidney medulla, kidney cortex, spleen, and bone marrow. Concentrations in the thyroid, spleen, and kidney probably included a substantial amount of free $^{125}$I and these values should be interpreted with caution. Significant free $^{125}$I was observed in blood, urine, faeces, and cage rinse samples following TCA precipitation, suggesting that a large portion of the $^{125}$I-rhPDGF-BB was degraded prior to excretion. In urine for example, less than 5% of the urinary radioactivity appeared to be bound based on TCA precipitation. This suggests nearly 95% of the radioactivity in urine was excreted as free $^{125}$I.

High concentrations of radioactivity were also present in the contents of the large intestine, bile, urine, and cecum contents, which suggested that biliary and renal excretion were the routes of elimination of rhPDGF-BB and/or free $^{125}$I. Recovery of radioactivity in urine averaged over 68% of the dose and the presence of 28% of the dose in faeces following IV administration are both consistent with this finding. Concentrations in the central nervous system tissues were the lowest among all tissues throughout the study and were near or baseline from 4 hours post-dose to the end of the study. Elimination was not complete at the
last time point (168 hours post-dose) and relatively high amounts of radioactivity were observed in the thyroid and skin at this time. However, these tissues are known to organify and/or otherwise retain free $^{125}$I, which was probably what the radioactivity represented in these tissues.

Based on autoradiographic images obtained from the 1 hour post-dose carcasses, high concentrations of radioactive material were seen in the tissues responsible for the elimination of the test article (i.e. liver, kidneys, and small intestine). This suggests that the test article in the blood was being transported to organs responsible for excretion and drug metabolism. By 4 hours post-dose, levels of radioactivity were significantly reduced in the blood, liver, and small intestine indicating that the test article was being removed from the blood and processed for excretion in the urine and faeces. This was also reflected in the large intestine and urinary bladder where higher levels of radioactivity were detected at the 4 and 8 hour post-dose time points.

**Conclusions**

The elimination of free $^{125}$I via the kidneys in the urine would follow after the proteolytic degradation of the protein test article in the liver or other tissues. A portion of the radioactive dose is also excreted as bound $^{125}$I in the faeces. Relatively high concentrations of radioactivity in liver, small intestine, and large intestine at the early time points in the QWBA study were observed, supporting roles for these organs in the elimination of the test article *in vivo*.

Based on the results from this study, it appears that the test article is widely distributed and cleared rapidly from the circulation. Radioactivity was excreted in urine and faeces primarily as unbound $^{125}$I with smaller amounts of bound $^{125}$I also excreted in faeces.

**2.6.4.3.2 Pharmacokinetics of $^{125}$I-rhPDGF-BB Following Intravenous Administration or Intramuscular Implantation in Combination with Augment Bone Graft β-TCP**

The results of the ADME study described above Section 2.6.4.3.1 demonstrated that systemic exposure to rhPDGF-BB is brief and that the protein is metabolised and excreted normally via renal and biliary routes. Following implantation of Augment at the surgical site, a limited
amount of rhPDGF-BB may gain access to the circulatory system leading to brief, but broad, systemic exposure. As a result, it is important to understand the pharmacokinetics of the protein once in the blood. To determine the circulating half-life of rhPDGF-BB administered intravenously compared to rhPDGF-BB combined with β-TCP implanted intramuscularly (Augment), BioMimetic has completed a study in a rat model.

This study is a follow-up study to BioMimetic’s ADME study “Tissue Distribution and Mass Balance of Radioactivity in Sprague-Dawley Rats Following Intravenous Injection of \( ^{125}\text{I}\text{-rhPDGF-BB} \)” - Study #1524-001.

The follow-up pharmacokinetics study (#1524-002) was designed to evaluate the kinetics, mass balance, and excretion of \( ^{125}\text{I}\text{-labeled rhPDGF-BB} \) administered as a single intravenous dose or as an intramuscular implant to male Sprague-Dawley rats. This study was conducted at MPI Research, in compliance with GLP for nonclinical Laboratory Studies. The implantation portion of this study was initiated on August 21, 2008; the IV portion of the study was initiated on August 26, 2008. The final report was completed in February 2009.

**Study Procedure**

This study was conducted to evaluate the pharmacokinetics, mass balance, and excretion of \( ^{125}\text{I}\text{-rhPDGF-BB} \) administered as a single intravenous dose or as an intramuscular implant in combination with a β-TCP matrix to male Sprague-Dawley rats. Two treatment groups of twenty-four (24) or thirty-six (36) male Sprague-Dawley rats were administered the test article via intravenous (IV) bolus or intramuscular (IM) implantation at an overall average dose level of 0.28 or 0.29 mg rhPDGF-BB/kg with 2.85 or 2.56 µCi \( ^{125}\text{I} \text{rhPDGF-BB/animal} \) (8.14 or 8.03 µCi/kg), respectively. The target dose volume for each group was 1.0 mL/kg (IV) or a standard dose of 0.3 mL (IM).

Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. Body weights were measured and recorded prior to dose administration. Blood samples were collected for whole blood radioactivity analysis at 1, 5, 10, 20 minutes, and 1, 4, 8, and 24 hours for animals dosed intravenously and additionally at 48, 72, 96, and 168 hours for animals that received intramuscular implants. Urine, faeces, and cage residue were collected at designated intervals up to 24 hours for
animals dosed intravenously and at 24 and 168 hours post-dose for animals who received intramuscular implantation for radioactivity concentration analysis. For animals in Group 2, that received the test article by intramuscular implantation, implant sites were harvested at 24 and 168 hours for analysis of radioactivity remaining in the tissues. The proportion of bound and unbound radioactivity was determined by trichloroacetic acid precipitation of blood, urine, faeces and cage rinse samples collected at designated intervals. The pharmacokinetic (PK) parameters $C_{\text{max}}$, $T_{\text{max}}$, terminal $t_{1/2}$, AUC$_{0-24}$, AUC$_{0-\infty}$, %F, CLs and CLs/F were determined using non-compartmental analysis for the test article administered either intravenously or implanted intramuscularly in combination with Augment β-TCP matrix from concentration-time data in the test species. Animals that did not have tissues collected were euthanized and the carcasses were discarded without further evaluation.

**Results**

Whole Blood Concentrations of Radioactivity: Whole blood concentration values are illustrated in Figure 2.6.4-6 following IV administration and Figure 2.6.4-5 following IM Implantation. Whole blood concentrations of radioactivity were measured at the time of euthanasia 1, 5, 10, 20 minutes, and 1, 2, 4, 8, and 24 hours after the intravenous dose for the animals of Group 1. For Group 2 animals, blood radioactivity was measured at the time of euthanasia 1, 5, 10, 20 minutes, and 1, 2, 4, 8, 24, 72, 96, and 168 hours following IM implantation.
Following IV administration of $^{125}$I-rhPDGF-BB, maximum concentrations of radioactivity in whole blood were observed at the first sampling time 1 minute ($T_{max}$) after the dose and declined rapidly through 20 minutes post-dose. The half-life ($t_{1/2}$) of the protein during the initial alpha phase in blood was 2.31 minutes, and the terminal $t_{1/2}$ over the gradual termination phase was 7.46 hours. Radioactivity in blood decreased more gradually from 1 hour through 24 hours after dose administration. Radioactivity in blood decreased through 24 hours to a level that was approximately 0.5% of the 1 minute $C_{max}$ level.

Following IM implantation of $^{125}$I-rhPDGF-BB combined with Augment $\beta$-TCP, relatively low amounts of radioactivity entered the circulation from the implant site compared to IV dosing. The maximum concentrations of radioactivity in whole blood were observed 8 hours ($T_{max}$) after dosing and declined gradually through 96 hours post-dose. The half-life ($t_{1/2}$) of the protein was calculated to be 30.30 hours. At 96 hours post-dose, radioactivity in blood was approximately 3% of the 8 hour $C_{max}$ level, and after 168 hours was below the limit of detection.

Recovery of Radioactivity in Excreta, Cage Residue, and Implant Sites Mean cumulative percent recoveries of radioactivity following IM implantation of $^{125}$I-rhPDGF-BB combined with Augment $\beta$-TCP expressed as a percentage of the administered dose in urine, faeces and cage residue are summarized in Figure 2.6.4-6. Recoveries were determined at a nominal sampling time 24 hours after IV dosing and expressed as a percentage of the administered dose.
Following intravenous administration of \(^{125}\text{I}-\text{rhPDGF-BB}\), cumulative recoveries of the administered radioactivity averaged 163.92% of the dose over the 24 hour study period. Renal excretion of the radioactivity averaged 100.27 ± 34.06%. An additional 17.87 ± 10.48% of the dose was recovered in faeces, 6.04 ± 0.85% was recovered in cage rinse, and 39.74 ± 47.64% in the carcass. A majority of the radioactivity associated with the test article was excreted in the urine. Based on the results of trichloroacetic acid (TCA) precipitation analyses of the urine samples, most of the excreted radioactivity was unbound. The radioactivity excreted in faeces, in contrast to the urine samples, appeared to be partially bound. Approximately 15% of radioactivity excreted in faeces was precipitated by the TCA procedure.
Following IM implantation of $^{125}$I-rhPDGF-BB combined with Augment $\beta$-TCP, cumulative recoveries of the administered radioactivity averaged 137.06% of the dose over the 168 hour study period. Renal excretion of the radioactivity averaged 109.83 ± 2.10%, and of that, 97.10% was recovered in the first 48 hours following dosing. An additional 17.75 ± 7.35% of the dose was recovered in faeces, 5.88 ± 0.73% was recovered in cage rinse, and 3.05 ± 2.99% in the carcass. Approximately 30% of the radioactivity remained at the implant site 24 hours after IM implantation and almost no radioactivity remained after 168 hours. A majority of the radioactivity associated with the test article was excreted in the urine, similarly to intravenous dosing. Based on the results of TCA precipitation analyses of the urine samples most of the excreted radioactivity was unbound (Figure 2.6.4-7). The radioactivity excreted in faeces, in contrast to the urine samples, appeared to be partially bound. The radioactivity at $T_{\text{max}}$ consisted of both bound (ranging from 34.56% to 69.39%) and unbound radiolabel, suggesting that the test article was partially degraded prior to entering the circulation after IM implantation.

**Conclusions**

The administration of $^{125}$I-rhPDGF-BB either by intravenous or intramuscular implantation routes leads to minimal systemic exposure in the blood and excretion primarily as unbound...
radioactivity in the urine and faeces. The systemic bioavailability of the test article was similar by both routes of administration since the %F was 88% when IM and IV dosing were compared. This suggests that IV dosing reasonably simulates exposure to rhPDGF-BB combined with Augment β-TCP administered by IM implantation. Entry of radioactivity into the circulation occurs slowly following IM implantation since the protein must pass through tissue prior to entering the bloodstream. Following IM implantation, the protein appeared to be partially degraded during its passage through the tissues prior to entering the circulation, since TCA precipitation of blood samples revealed that there is a mix of bound and unbound radioactivity present. These data suggest that the test article is rapidly released from the β-TCP matrix over the 24 hours following implantation, and is nearly depleted from the implant site by 168 hours post-dose.

2.6.4.3.3 Pharmacokinetics of Recombinant Human Platelet-Derived Growth Factor-BB (rhPDGF-BB) in Sprague-Dawley Rats Following Intravenous Administration of rhPDGF-BB

BMTI carried out a non-GLP study to evaluate the pharmacokinetics of rhPDGF-BB administered as a single intravenous dose to male Sprague-Dawley rats.

Study Procedure

Forty-eight (48) male Sprague-Dawley rats with an average weight of 227 grams were randomly distributed into two groups with each group containing 24 animals. Group 1 animals received a single intravenous average bolus dose of 440 μg/kg rhPDGF-BB in a target volume of 1.1 mL/kg. Group 2 consisted of 24 male Sprague-Dawley rats that received a single intravenous bolus dose of 20 mM sodium acetate in a target volume of 1.1 mL/kg.

Serum samples were collected from six animals per time point per group at baseline, 1, 5, 10, and 20 minutes, 1, 4, 8, 24, 48, 72, 96 and 168 hours post dose for all animals (Groups 1 and 2). The quantification of rhPDGF-BB in the serum collected was conducted using the Quantikine ELISA kit from R&D systems. Pharmacokinetic modelling was performed using a non-compartmental module of WinNonlin. Parameters calculated were: T_{max}, C_{max}, AUC_{0-\text{last}} and CL. Statistical analysis was limited to descriptive parameters such as means, standard deviations, and coefficient of variation, as appropriate.
Results
The mean serum rhPDGF-BB concentration-time values are illustrated in Figure 2.6.4-8. A decrease in concentration of rhPDGF-BB in serum between 5 minutes and 1 hour was observed. From 1 hour to 168 hours the concentration of rhPDGF-BB in serum was below the level of quantification (<0.156 ng/mL) for the ELISA.

Figure 2.6.4-9: Mean serum rhPDGF-BB concentration values following IV administration.

Table 2.6.4-5 represents the pharmacokinetic analysis of the parameters for IV dosing of rhPDGF-BB in male Sprague-Dawley rats. The average dose delivered was 440 μg/kg. $T_{\text{max}}$ was observed at 0.0167 hours (1 minute) with a $C_{\text{max}}$ at 6161.2 ng/mL. The $\text{AUC}_{0-\text{last}}$ was 375.64 hr*ng/mL and the clearance (CL) was 17.5 mL/min/kg.

<table>
<thead>
<tr>
<th>Route</th>
<th>Dose (μg/kg)</th>
<th>$C_{\text{max}}$ (ng/mL)</th>
<th>$T_{\text{max}}$ (hours)</th>
<th>$\text{AUC}_{0-\text{last}}$ (hr*ng/mL)</th>
<th>CL (mL/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>440</td>
<td>6161.2</td>
<td>0.0167</td>
<td>375.64</td>
<td>17.5</td>
</tr>
</tbody>
</table>

Conclusions
The intravenous (IV) administration of rhPDGF-BB resulted in a high initial systemic exposure. The molecule was rapidly eliminated from the blood over the first 10 minutes after dosing (Ossenberg, 1974).
2.6.4.4 Discussion and Conclusions

In comparing the data from the ADME and pharmacokinetics studies, several common trends with regard to the kinetics, metabolism and excretion of $^{125}$I-rhPDGF-BB in rats can be highlighted.

- Systemic exposure to rhPDGF-BB is minimal and brief following administration by intravenous or intramuscular implantation routes.
- Systemic bioavailability of rhPDGF-BB is similar following either intravenous or intramuscular implantation routes.
- Excretion of radioactivity is mainly in the urine, and is almost completely unbound. Radioactivity excreted in the faeces is mostly unbound along with smaller quantities of bound radiolabel.

The ADME and PK studies were conducted independently of one another. The trends in the data led to similar conclusions regarding kinetics and excretion of rhPDGF-BB. These studies provide further support for the safety of Augment showing that administration of rhPDGF-BB leads to brief and minimal systemic exposure.