Lack of Efficacy of the Combination of Pamidronate and Vitamin D on Regression of Prostate Cancer in the Dunning Rat Model

Paula Herring,¹ Jesse Ingels,¹ Laura D. Carbone,¹ Karen D. Barrow,¹ David Osborn,² Dennis J Dietzen,³ & Linda Pifer¹

¹Department of Medicine, University of Tennessee Health Sciences Center, Memphis, Tennessee; ²G.I. Pathology Partners/Pathology Partners of Memphis, Memphis, Tennessee; ³Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri

Address all correspondence to Paula Herring, MD, University of Tennessee, 956 Court Avenue, Room G326 Coleman Building, Memphis, TN 38163, phone: (901) 448-5774, fax: (901) 448-7265

Dear Editor:

Beneficial effects of bisphosphonates on metastatic disease from prostate carcinoma have been reported.¹ In the Dunning rat prostate model system, 1α, 25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] has also shown efficacy in inhibiting the primary tumor volume and in reducing the number and size of lung metastases.² However, concerns about inducing hypercalcemia and hypercalciuria in cancer patients have limited the use of [1,25(OH)₂D₃] by itself.³ We investigated whether the combination of pamidronate, a bisphosphonate used to treat hypercalcemia of malignancy, with [1,25(OH)₂D₃], a vitamin D analog, had efficacy in reducing primary tumor burden and lung metastases and altering cytokine profiles in the Dunning rat prostate model.

Forty-eight inbred Copenhagen rats 4–6 weeks old were injected in the subcutaneous layer of the right flank with 5 × 10⁵ MATLyLu cells, a rat prostate cancer cell line shown to produce local soft tissue tumors and metastatic lesions to the lymph nodes and lungs of male rats (Dunning model).⁴ Primary prostatic tumors were measured at the end of 2 weeks, when the rats were divided into four groups of 12 animals each with equal tumor size. Intraperitoneal injections were administered three times per week for 2 weeks, with Group 1 receiving [1,25(OH)₂D₃] 100 ng, Group 2 receiving drug vehicle, Group 3 receiving a combination of [1,25(OH)₂D₃] 100 ng plus pamidronate 1 mg, and Group 4 receiving only pamidronate 1 mg. At the end of 2 weeks, the tumors were excised; the primary tumor volume and the histopathological evidence of lung metastases were assessed. One lung slice/rat was examined. The pathologist did not know the source of the lung examined. Laboratory studies included serum BUN, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), calcium, and cytokine levels ( interleukin 6, interleukin 1 beta, and interferon gamma).

An additional 30 inbred Copenhagen rats 4–6 weeks old were used to determine the effect of treatment on survival. They were divided into four groups, which were given intraperitoneal injections three times per week for 2 weeks and treated in the same way as the experimental groups: Group 1 received [1,25(OH)₂D₃] 100 ng, Group 2 drug vehicle, Group 3 a combination of [1,25(OH)₂D₃] 100 ng plus pamidronate 1 mg, and Group 4 only pamidronate 1 mg.

Rats in this study were sacrificed when any signs of respiratory distress were present. All studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

All statistics except the survival analysis were done using analysis of variance (ANOVA). To determine the effects of treatment arm on overall survival, a time to event analysis was used. All statistics were done using the SAS System for Windows (version 8.1).

The biochemical results are summarized in Table 1. There were no differences among the groups with respect to serum creatinine, AST, ALT, and BUN. On the other hand, calcium levels were higher in Group 1 ([1,25(OH)₂D₃] 100 ng) compared to Group 3 (a combination of [1,25(OH)₂D₃] 100 ng plus pami-
Table 1. Means ± Standard Deviations of Laboratory Measures Following Treatment

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>0.7 ± 0.2 (n = 9)</td>
<td>0.6 ± 0.1 (n = 9)</td>
<td>0.6 ± 0.1 (n = 9)</td>
<td>0.6 ± 0.1 (n = 10)</td>
<td>0.49</td>
</tr>
<tr>
<td>AST</td>
<td>274.3 ± 146.1 (n = 8)</td>
<td>215.2 ± 84.6 (n = 9)</td>
<td>242.4 ± 117.0 (n = 9)</td>
<td>187.2 ± 57.6 (n = 10)</td>
<td>0.35</td>
</tr>
<tr>
<td>ALT</td>
<td>38.9 ± 27.8 (n = 8)</td>
<td>83.1 ± 31.4 (n = 9)</td>
<td>40.8 ± 31.8 (n = 9)</td>
<td>26.2 ± 6.5 (n = 10)</td>
<td>0.48</td>
</tr>
<tr>
<td>BUN</td>
<td>25.4 ± 17.0 (n = 9)</td>
<td>82.0 ± 18.9 (n = 9)</td>
<td>48.6 ± 44.6 (n = 9)</td>
<td>23.1 ± 8.8 (n = 10)</td>
<td>0.16</td>
</tr>
<tr>
<td>Calcium</td>
<td>11.0 ± 1.1 (n = 9)</td>
<td>10.6 ± 1.0 (n = 9)</td>
<td>9.6 ± 0.5 (n = 9)</td>
<td>9.7 ± 0.2 (n = 10)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Interleukin 6</td>
<td>788.4 ± 72.1 (n = 9)</td>
<td>892.9 ± 83.1 (n = 10)</td>
<td>1803.0 ± 1259.2 (n = 8)</td>
<td>899.6 ± 23.6 (n = 9)</td>
<td>0.004</td>
</tr>
<tr>
<td>Interleukin 1B</td>
<td>142.1 ± 207.7 (n = 9)</td>
<td>169.5 ± 164.1 (n = 10)</td>
<td>89.6 ± 111.4 (n = 8)</td>
<td>75.8 ± 66.1 (n = 9)</td>
<td>0.50</td>
</tr>
<tr>
<td>Interferon gamma</td>
<td>18.8 ± 7.2 (n = 9)</td>
<td>17.2 ± 4.4 (n = 10)</td>
<td>26.9 ± 4.8 (n = 8)</td>
<td>70.3 ± 74.7 (n = 9)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Pamidronate 1 mg and Group 4 (pamidronate 1 mg) (p = 0.0004 and p = 0.0005, respectively). The calcium levels of Group 2 were also statistically significantly higher than Group 3 (p = 0.0075) and Group 4 (p = 0.0095).

Interleukin 6 and interferon gamma levels differed significantly among the treatment groups, with interleukin 6 levels higher in Group 3 (combination of 1,25-dihydroxyvitamin D₃ 100 ng and pamidronate 1 mg) relative to all other groups (p = 0.0013 vs. Group 1, p = 0.0028 vs. Group 2, and p = 0.0036 vs. Group 4), and interferon gamma levels higher in Group 4 (pamidronate 1 mg) relative to all other groups (p = 0.01 versus Group 1, p = 0.004 vs. Group 2, and p = 0.02 vs. Group 3).

Interleukin 1 beta levels did not differ among the groups (p > 0.05).

Pathological findings: There were no differences among the groups with respect to the primary prostatic tumor weight (p > 0.05), the presence or absence of metastatic lesions to the lungs (p > 0.05), or the absolute number of metastatic lung lesions (p > 0.05), although the number of rats with metastatic lung lesions as well as the absolute number of metastatic lesion was small (n = 11 and n = 35, respectively). In addition, there was no effect of treatment group on overall survival time.

In our study, there was upregulation of interferon gamma levels, a cytokine, which has been associated with antitumor responses in the group treated with pamidronate alone. In contrast, the group treated with the combination of pamidronate with [1,25(OH)₂D₃] had increased levels of interleukin 6, a cytokine that has been associated with tumor promotion. Despite these differences in cytokine profiles among the groups, we found no effect of therapy with [1,25(OH)₂D₃], pamidronate, or the combination of [1,25(OH)₂D₃] with pamidronate at reducing the primary prostatic tumor burden and metastatic lung disease, or improving the overall survival in the Dunning rat model.

The combination of pamidronate with vitamin D at the doses used in our study is not effective at ameliorating prostate cancer in the Dunning rat model. In contrast, monotherapy with pamidronate was associated with potentially favorable effects on the cytokine profiles in this model and merits further study.

REFERENCES