BIOLOGICAL EVALUATION OF NEW VITAMIN D₂ ANALOGUES:

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Introduction

1,25-dihydroxyvitamin D₃ (1,25D) is a well-known anti-rachitic agent that has also been shown to regulate cellular differentiation, proliferation, and immunomodulation. The biological responses to 1,25D are mediated by binding to the vitamin D receptor (VDR), which then forms a heterodimer with retinol X receptor (RXR). Together this complex binds to vitamin D response elements (VDREs) in the promoter region of target genes. CYP24A1, an enzyme responsible for degradation of 1,25D, is the most strongly regulated gene by VDR. Another VDR-target gene is a monocytic cell differentiation marker CD14, a co-receptor for bacterial lipopolysaccharide characteristic for monocytes and macrophages. Due to the expanded magnitude of 1,25D activity, it therefore suggests its potential therapeutic applications for the treatment of several diseases and disorders, including hyperproliferative diseases, immune dysfunctions, endocrine disorders, and metabolic bone diseases. However, therapy using natural vitamin D hormone, 1,25D, has been impeded in most cases due to its potent calcemic activity. Current research is focused on developing analogues with selective properties including improved anti-proliferative and pro-differentiating activities, as well as lower calcemic effects. In this study, we described the activities of a new set of analogues, compared to the one of PRI-1907 and to the 19-nor analogs of 1,25-dihydroxyvitamin D₃ with the natural or 24-epi side-chain, PRI-5100 (paricalcitol) and PRI-5101, respectively.

Methods

Serum Calcium: Quantification and weight measure

The analogues were each weighed in batches of 5 µg and resuspended appropriately (3 µg/kg) every other day for three weeks. Calcium measurement was determined daily by the last day using QuantiChrom Calcium Assay Kit. Weight was checked once over a week.

Preparation and incubation of cell culture

Monocytic cell lines (THP-1) were cultured in a 100-mm diameter tissue culture plate of cell surface markers CD14 and CD45 and analysis using the FACS. Cytometry flow (Becton-Dickinson, San Jose, CA). Cells analysis was performed using flow software.

Western Blotting

100 µg of total cell protein (extracted from 5 x 10⁶ cells) and transferred to PVDF membrane. The membranes were incubated with a primary antibody, and a fluorescent secondary antibody. The protein bands were visualized with chemiluminescence.

Statistical analysis

All data were described as mean ± standard deviation (SD). All experiments were carried out in triplicate. Student's t-test and analysis of variance (ANOVA) were used to assess differences among groups, and p values were set at 0.05.

Results

Relative binding affinities of 1,25D and analogues for human VDR

<table>
<thead>
<tr>
<th>Binding Affinity</th>
<th>1,25D</th>
<th>PRI-1907</th>
<th>PRI-5100</th>
<th>PRI-5101</th>
<th>PRI-5201</th>
<th>PRI-5202</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 nM</td>
<td>100</td>
<td>38.6</td>
<td>41.35</td>
<td>47.14</td>
<td>194.46</td>
<td>64.4</td>
</tr>
</tbody>
</table>

CYP24A1, VDR's most highly regulated gene, is greatly upregulated in the leukemic HL60 cell line following treatment with analogues.

CD14, A co-receptor for the detection of bacterial lipopolysaccharide (LPS), is upregulated in the leukemic HL60 cell line, following treatment with analogues.

All analogues have lower calcemic activities in mice compared to 1,25D.

References

(References to be included here)

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