MEK inhibitors in orthopaedics: applications in NF1

Little DG1,2, El-Hoss J1,2, Deo N1,2, Mikulec K1,2, McDonald M1,2, Schindeler A1,2
1 Orthopaedic Research & Biotechnology, The Kids Research Institute at The Children’s Hospital at Westmead
2 Discipline of Paediatrics and Child Health, The University of Sydney, Sydney, Australia

Conclusions

Congenital tibial dysplasia (CTD) is often associated to the genetic disorder of type I neurofibromatosis (NF1). Patients suffering from CTD display bowing of the tibiae, and are prone to fracture and progression to pseudarthrosis (non-union).

To date, therapies for CTD are very limited, and amputation at a young age is not an uncommon outcome for this condition. Recent genetic data suggests that local double-inactivation of the NF1 gene may be implicated in CTD pathobiology, resulting in over-activation of the Ras-MAPK pathway.

MEK inhibitor treatment during fracture healing promoted cartilage formation and delayed resorption. Combination treatment of PD0325901 and rhBMP2 synergistically promoted bone formation and fibrous tissue accumulation, but was no more effective than rhBMP2 at healing a pseudarthrosis.

Type I Neurofibromatosis is a complex disorder that impacts the nervous and musculoskeletal tissues. It is associated with mutations in the NF1 gene, resulting in over-activation of the Ras-MAPK pathway. PD0325901 is a new MEK inhibitor capable of limiting Ras-MAPK activation, and has been trailed in NF1 mouse models to treat neural lesions and leukemia (1,2). A sister compound (AZD6244, Selumetinib, AstraZeneca), is also under clinical trials in a pediatric NF1 population.

Congenital Tibial Dysplasia (CTD) is a severe complication that is associated to NF1, and results in a persistent non-union. In severe cases, a pseudarthrosis can develop with the accumulation of a highly fibrotic fracture callus. We recently developed a new model of NF1 pseudarthrosis that allows preclinical testing of novel compounds (3).

In this study, we hypothesized that the MEK inhibitor PD0325901 could rescue the fibrosis in our fracture model. However, the impacts of MEK inhibitors PD0325901 or AZD6244 have never been described in fracture healing. Therefore, we performed an experiment to determine the impact of PD0325901 and AZD6244 during fracture healing. We then performed a second experiment to determine if PD0325901 in combination with rhBMP2 could prevent fibrosis in our NF1 fracture model.

Impact of PD0325901 and AZD6244 in wild-type mice

To determine the impact of PD0325901 and AZD6244 during fracture healing, C57Bl/6 mice underwent a closed tibial fracture. Mice were treated during the different stages of fracture healing as described in Figure 1.

We found that at day 10, PD0325901 significantly promoted cartilage deposition, while AZD6244 only showed a trend (Figure 1A, B). If treatment was stopped at day 10, the cartilage was remodelled by day 21, and we saw no change in osteoclasts (Figure 1, Early group). If treatment was started at day 10, cartilage resorption was delayed, and osteoclast surface was also reduced at day 21 (Figure 1, Late group).

Taken together, these data suggest that MEK inhibitor treatment can promote cartilage formation, delay cartilage resorption, and reduce osteoclast surface in fracture healing.

We used a previously published NF1 fracture model to determine if PD0325901 could be used to treat or prevent a NF1 pseudarthrosis (3). NF1 mice were treated with a Cre expressing adenovirus at the site of an open fracture to induce a pseudarthrosis. PD0325901 was administered alone at 10 mg/kg/day for the first 10 days of healing or in combination with local rhBMP2 (10µg). Mice were harvested at day 21 to assess fracture union rates, histology, and bone volume.

Table 1 - Union rates for treatment of NF1 mice with a pseudarthrosis

<table>
<thead>
<tr>
<th>Unions</th>
<th>Partial unions</th>
<th>Non-unions</th>
<th>Total N</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0 (0%)</td>
<td>5 (36%)</td>
<td>9 (64%)</td>
<td>14 -</td>
</tr>
<tr>
<td>PD0325901</td>
<td>1 (8%)</td>
<td>4 (31%)</td>
<td>8 (61%)</td>
<td>13 1</td>
</tr>
<tr>
<td>+ rhBMP2</td>
<td>9 (69%)</td>
<td>2 (15%)</td>
<td>2 (15%)</td>
<td>13 0.0002*</td>
</tr>
<tr>
<td>PD0325901</td>
<td>12 (80%)</td>
<td>2 (13%)</td>
<td>1 (7%)</td>
<td>15 0.0001*</td>
</tr>
</tbody>
</table>

Table 2 (1) - MicroCT analysis showed that rhBMP2 treatment significantly increased bone volume compared to vehicle by 3-fold (Figure 2). Combination rhBMP2 + PD0325901 acted synergistically to promote bone formation in NF1 pseudarthrosis; it promoted 6-fold increase in bone formation compared to vehicle, and 2-fold over rhBMP2 alone. While we observed a change in bone volume, there was also an increase in callus volume resulting in a decrease in bone volume over tissue volume in the rhBMP2 and combination treatment (data not shown). Furthermore, no treatment resulted in a change in fibrous tissue accumulation this model of NF1 pseudarthrosis (Figure 3).

PD0325901 and AZD6244 are a growing class of compounds to treat cancer. Yet, little information is available regarding their impacts on the skeletal or during fracture healing. In this study, we showed that MEK inhibitor treatment during fracture healing could promote cartilage deposition in the early phase of fracture healing, and delay cartilage resorption. We also found that osteoclasts surface was reduced, yet this was reversible.

In NF1 pseudarthrosis, treatment with PD0325901 did not increase union rate, suggesting that other pathways are involved. Combination treatment with rhBMP2 synergistically increased bone volume and fibrous tissue in an NF1 calvarial.