RAP-011 Augments Callus Formation in Closed Rat Fractures
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Introduction
RAP-011 is a soluble chimeric protein consisting of extracellular domain of the Activin Type 2a receptor (ActRIIA) fused to a murine IgG2a-Fc. Antagonism of activin signaling with this compound has been explored as an effective treatment for osteoporosis. When administered to OVX mice, RAP-011 increased bone formation as well as load and energy to failure, with positive effects noted as early as 2 weeks and maximal at 6-12 weeks [1]. This was associated with increases in trabecular number and thickness, as well as increases in serum markers of bone formation and decreases in markers of bone resorption. In a phase 1 randomized, double-blind, placebo-controlled study, the human counterpart, ACE-011 also was shown to increase bone formation markers and decrease markers of bone resorption [2]. ACE-011 was also shown to circulate for relatively long periods of time (mean half-life of 24-32 days). In this study, we sought to investigate the potential of RAP-011 as an anabolic agent for fracture repair.

Hypothesis
We hypothesized that RAP-011 would increase net callus formation in a rodent model of closed fracture healing.

Methods
Animal Surgery: Rat studies were carried out with permission from the local institutional animal ethics committee. Closed femoral fractures were created in 66 male Wistar rats by three point bending using an Einhorn apparatus, with six rats lost during surgery. Rats were given twice weekly subcutaneous (s.c.) injections of RAP-011 (10mg/kg) or phosphate buffered saline vehicle. Endpoints were at 2, 4, and 6 weeks and operated and contralateral limbs were harvested.

Radiological analyses: Fractured limbs were x-rayed (Faxitron) before being fixed in 4% PFA and transferred to 70% ethanol. X-ray images were analyzed for union in terms of both cortices and callus length (Figure 1). Bones were then scanned by QCT (Stratec) with measured parameters including bone mineral density (BMD), bone mineral content (BMC), bone volume (BV), and periosteal and endocortical circumferences. MicroCT scanning (Skyscan) was then performed in order to generate illustrative images.

Results
RAP-011 was well tolerated by rats and no complications or side effects were noted with RAP-011 dosing. Initial visual inspection of healing fractures suggested an increased callus length that was confirmed by measurement. Callus length was increased by 32% in RAP-011 treated animals at 2 weeks (P<0.01), increased by 18% at 4 weeks (P<0.05), and increased by 16% at 6 weeks (P<0.01). No significant change was observed in the initial union rate by x-ray grading.

It has been previously shown that RAP-011 treatment can have systemic effects [1] and this was confirmed by QCT in the contralateral limb. No significant effects were seen at weeks 2 or 4, but small increases in parameters including total bone volume (8%, P<0.05) and periosteal perimeter (4%, P<0.05) were seen at week 6. At the 6 week time point significant changes were seen in callus properties, particularly BMC and total callus volume (Figure 2). BMC was increased by 31% (P<0.01) and callus volume by 36% (P<0.01), which led to a calculated 93% increase in polar moment of inertia.

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Conclusion
RAP-011 and its human analogue ACE-011 have been previously shown to increase BMD in the context of normal bone homeostasis, but this is the first study to examine the use of an ActRIIA decoy receptor-antibody fusion in the context of fracture repair. At 6 weeks we observed significant increases in fracture callus length, volume, BMC, and calculated strength. A previous study showed increases of callus BMC of 14% with 5μg/kg/day and 31% with 30μg/kg/day PTH (1-34) [3], which is the most potent systemic bone anabolic in clinical use. The current study result of a 31% increase with RAP-011 is thus equivalent to a moderate to high dose of PTH(1-34).

Figure 1. Week 2 femoral fracture schematically showing callus length as the average of both sides of a 2D x-ray callus relative to femur length.

Figure 2. QCT parameters of fractured femora: changes with RAP-011

Figure 3. MIP reconstructed microCT images of representative calluses of femoral fractures treated with Vehicle or RAP-011 at 6 weeks.

Histology and histomorphometric analyses will be performed to elucidate the effects of RAP-011 in the early and late stages of repair.

References

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