The effect of a single Parathyroid Hormone (PTH) injection on the healing of stress fractures.

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Stress fractures:

- Stress fractures (SFx) result from cyclic repetitive loading.
- Common in athletes, dancers and recruits (Kiuru et al., 2005).
Are stress fractures significant:
Stress fractures can lead to serious fractures, an example of that is **Atypical Femoral Fractures (AFF)** in Bisphosphonate treated patients.

(Girgis and Seibel, 2011)
Intermittent PTH administration:

- Creates an anabolic action
- Increase in trabecular bone formation
- Increased osteoblast activity (Hock, 1999)
- Inhibition of osteoblastic apoptosis (Jilka et al., 1999).

(Dempster et al., 1993; Finklestein et al., 1996)
The aim of our study was to determine if a single treatment with PTH, 24 hours following stress fracture induction, will accelerate histomorphometric indices of stress fracture healing.
Significance:

PTH treatment is costly $6,000 – $7000 / per year.

A single injection is more acceptable to patients than multiple.

Minimises potential adverse events eg prostate cancer and Osteosarcoma in rats (Schneider et al., 2005; Tashjian et al., 2006).

A single PTH injection (if proven effective) could also help athletes return back to training as quick as possible.
Experimental design:

16 female wistar rats 300 g had an ulnar stress fracture induced, according to the model created in Forwood’s lab by Kidd et al., 2010.
**Groups:**

<table>
<thead>
<tr>
<th>Group</th>
<th>2 weeks cohort</th>
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<tbody>
<tr>
<td>PTH Group</td>
<td>Single PTH injection (n=8).</td>
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<tr>
<td>VEH Group</td>
<td>Single VEH injection (n=8).</td>
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**PTH groups** received a single Human PTH-(1-34) peptide (Sigma-Aldrich) dissolved in 0.9% saline with 1% rat heat-inactivated serum in a final volume of 200 μl and injected s.c. 8 μg/100g – 24 hours after stress fracture loading.

**VEH groups:** received an equivalent single saline vehicle injection – 24 hours after stress fracture loading.
Two toluidine blue-stained and two TRAP-stained sections from each bone were measured at three standard levels along the stress fracture:

1- Medial end: closer to the medullary cavity.
2- Middle point: half-way between the cortical margin and the medullary cavity of the bone in transverse section.
3- Lateral end: closer to the cortical margin.
Osteomeasure™ histomorphometric analysis:

**Red**  Cortical area (Ct.Ar, mm\(^2\))

**Yellow**  Woven bone area (Wo.B.Ar, mm\(^2\))

**Light blue**  Woven bone Width (Wo.B.Wi, mm)

**Dark red**  Length of stress fracture (SFx.Le, μm)

**Green**  Number of osteoclasts (N.Oc)

**Yellow**  Osteoclast surface perimeter (OC.Pm, μm)
<table>
<thead>
<tr>
<th>Color</th>
<th>Measurement Description</th>
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<tbody>
<tr>
<td>Blue</td>
<td>Porosity area (SFx.Po.Ar, µm²)</td>
</tr>
<tr>
<td>Light Blue</td>
<td>Erosion area (SFx.E.Ar, µm²)</td>
</tr>
<tr>
<td>Yellow</td>
<td>Length of remodelling unit along fracture line (BMU.Le, µm)</td>
</tr>
<tr>
<td>Purple</td>
<td>Area of healed new bone (SFx.He.Ar, µm²)</td>
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From the standard variables measured, four derived variables were calculated:

1- Number of osteoclasts per \( \mu \text{m}^2 \) of Basic Multicellular Unit (BMU) area.

2- Number of osteoclasts per \( \mu \text{m} \) of Basic Multicellular Unit (BMU) length.

3- Percentage healing = \( \frac{\text{Healing area}}{\text{Porosity (BMU) area}} \) \times 100

4- Woven bone apposition rate per day = \( \frac{\text{Wo.B width} \times \text{Wo.B perimeter}}{\text{Number of days (14)}} \)
Results:

2 weeks cohort:
Medial end (SFx):
There were no significant differences between PTH and VEH.

Middle point (SFx):

There was a significant difference between PTH and VEH groups in terms of Number of osteoclasts per µm of Basic Multicellular Unit (BMU) length.

† Marginally significant (Mean difference > 2 X SEM)
Lateral end (SFx):
There were significant differences between PTH and VEH groups in terms osteoclasts number, BMU length and porosity area perimeter.

* Significant P<0.05
Combining all measurements from the three points along the stress fracture (SFx) showed that PTH group has a significantly higher woven bone area, BMU length and porosity area perimeter.

† Marginally significant (Mean difference > 2 X SEM)
* Significant P<0.05
Discussion:

- Stress fractures heal by direct remodelling (*Kidd et al.*, 2010).
- Basic Multicellular Units (BMU) are formed in the first 2 weeks.
- Osteoclasts play a significant role.
Discussion:

- Significant increase in the number of osteoclasts along the SFx
- Explained by PTH induction of monocyte chemotactic protein-1 (MCP-1).
- Responsible for differentiation and recruitment of osteoclasts precursors in early remodeling phases *(Wu et al., 2013).*
Conclusions:

1- Healing of stress fractures starts from the medullary cavity (medial end) and progresses towards woven bone (lateral end) of stress fracture (SFx).

2- A single PTH injection 24 hours after SFx results in active changes in the dynamics of bone remodeling after 2 weeks.

3- PTH increases the number of osteoclasts, BMU length and porosity area perimeter towards the lateral end of stress fracture (SFx).
Future research:

1- Investigation of the effect of a single PTH injection on the healing of stress fractures (SFx) after 3 days, 1 week, 6 weeks and 10 weeks.

2- Investigation of the effect of a daily PTH injection (for 14 days) on the healing of stress fractures (SFx).

3- Investigation of the efficacy of daily PTH treatment for two weeks on acceleration of the indices of SFx healing in the presence of a concurrent Bisphosphonanate treatment (Alendronate).
Our team:

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HMM...YES, MR. BAKER, I'D SAY YOU HAVE A STRESS FRACTURE...
References:


• Tashjian AH Jr, Gagel RF. Teriparatide [human PTH(1-34)]: 2.5 years of experience on the use and safety of the drug for the treatment of osteoporosis. J Bone Miner Res. 21:354–436, 2006

• Wu AC, Morrison NA, Kelly WL, Forwood MR. MCP-1 expression is specifically regulated during activation of skeletal repair and remodeling. Calcified Tissue Int. 92:566-575, 2013.