Repository Corticotropin Injection (H.P. Acthar® Gel) Inhibits Bone Degradation in Rat Adjuvant-Induced Arthritis

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Abstract

Repository corticotropin injection (RCI: H.P. Acthar® gel) contains a purified porcine pituitary ACTH-analogue, and is an FDA-approved treatment for short-term adjunctive therapy of acute episode or exacerbation in rheumatic disorders. It has been suggested that ACTH modulates the immune response via binding to melanocortin receptors (MC1R to MC5R). These receptors are expressed on a number of cells including immune cells and bone cells, and have been shown to modulate several immune responses. Treatment with RCI showed significant and dose responsive inhibition of disease induction as determined by evaluation of ankle diameter (area under the curve (AUC) inhibition of 44%, 74%, 94% for 40 IU/kg, 160 IU/kg and 400 IU/kg, respectively) whereas prednisolone reduced swelling by 33%. Microscopic examination of the ankle joints showed that RCI significantly inhibited total histopathology sum score by 64% and 85% at 160 IU/kg and 400 IU/kg, respectively, while treatment with prednisolone resulted in 33% inhibition, which was not significantly different from the controls. Furthermore, RCI inhibited inflammation-related bone resorption and reduced the number of osteoclasts in the inflamed joint. Interestingly, prednisolone significantly reduced the number of osteoclasts but did not show a significant benefit on joint damage as evaluated by bone resorption and cartilage damage. RCI treatment significantly inhibited the development of disease in rat AIA, whereas prednisolone treatment alone only showed a minor benefit. Furthermore, there was a significant reduction in the number of osteoclasts following RCI treatment. These findings support the use of RCI for treatment of rheumatoid arthritis and suggest a potential bone sparing effect.

Study Objective

To investigate the immune modulatory effect of H.P. Acthar® gel in a rat adjuvant-induced arthritis model and the affect on osteoclast-mediated bone resorption compared to an immunosuppressive steroid.

Methods

- Male Lewis rats were weighed and randomized by body weight on study day 0. Animals were administered 0.1 ml of Freund's incomplete adjuvant with supplemented with lipoidal amine (6 mg in 100µl) by intradermal injection. Dosing began 2 hours post disease induction.
- On day 7, ankles calipered to determine baseline measurements. Animals will be calipered and weighed on study days 9-15.

Study Design

Figure 1. Study Design and Assessments

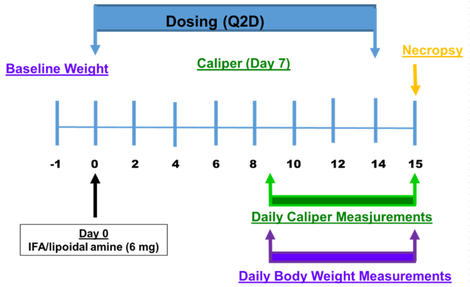


Table 1. Treatment Groups

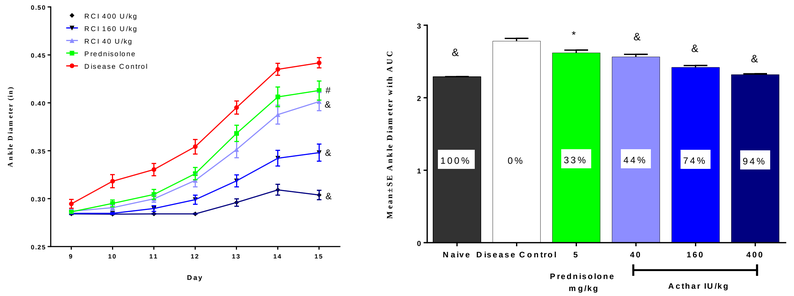
N	Treatment	Dose Level	Regimen
4	Naive	N/A	N/A
8	Disease + RCI	5 ml/kg	Q2D (Days 0-14)
8	Disease + Prednisolone	5 mg/kg	Q2D (Days 0-14)
8	Disease + RCI	40 IU/kg	Q2D (Days 0-14)
8	Disease + RCI	160 IU/kg	Q2D (Days 0-14)
8	Disease + RCI	400 IU/kg	Q2D (Days 0-14)

Study Assessments

- In-life
  - Body weight
  - Ankle diameter
- Terminal
  - Paw Weight
  - Histological analysis was performed on paraffin embedded ankles. Sections were stained with toluidine blue and scored for inflammation, pannus, cartilage dames, bone resorption, periosteal bone formation and osteoclast numbers by a certified pathologist on a scale of 0 (normal) to 7 (severe).

Results

Figure 2. Anti-inflammatory effect of RCI on AIA in rats.



Effect of RCI on paw swelling (A), ankle diameter AUC (B) in a rat model of AIA. Freund's incomplete adjuvant supplemented with lipoidal amine except in the normal group, was injected at the base of the tail in male Lewis rats. Placebo control (5 ml/kg), RCI (40, 160, or 400 IU/kg) or prednisolone (5 mg/kg) was administered by subcutaneous injection EOD for 7 days starting the day of adjuvant injection. Treatment with RCI in a dose responsive manner significantly inhibited ankle diameter. Statistical analysis was performed by 2-way ANOVA for ankle diameter or 1-way ANOVA for the ankle diameter AUC with a Holm-Sidak post-hoc analysis . *P < 0.05, $^{\#}P < 0.001$,$^{\&}P < 0.0001$ significant difference from disease control group.

Table 2. Histopathology Scoring

Treatment	Inflammation	Pannus	Cartilage Damage	Bone Resorption	Periosteal Bone Formation	Osteoclast Numbers
Disease	5.56 ± 0.11	0.97 ± 0.16	0.97 ± 0.16	4.81 ± 0.09	1.03 ± 0.24	14.04 ± 0.95
RCI 40 IU/kg	4.13 ± 0.38	0.66 ± 0.08	0.66 ± 0.08	2.50 ± 0.52*	0.69 ± 0.22	6.75 ± 0.84*
RCI 160 IU/kg	2.56 ± 0.24*	0.34 ± 0.08*	0.44 ± 0.06*	1.22 ± 0.29*	0.19 ± 0.06*	2.82 ± 0.58*
RCI 400 IU/kg	1.41 ± 0.13*	0.03 ± 0.03*	0.06 ± 0.04*	0.44 ± 0.26*	0 ± 0*	0.70 ± 0.34*
Prednisolone	4.06 ± 0.32	0.59 ± 0.08	0.59 ± 0.08	3.28 ± 0.23	0.38 ± 0.05*	8.67 ± 0.93*

RCI suppresses joint inflammation and joint destruction in rats with adjuvant-induced arthritis (AIA). Summed ankle histopathology scores were significantly reduced by treatment at 160 IU/kg (64% reduction) or 400 IU/kg (85%). This response appears to be primarily driven by the reduction in inflammation of 26%, 54%, or 75% and a decrease in bone resorption of 48% reduction, 75%, or 91% with treated with RCI at 40, 160, and 400 IU/kg, respectively, as compared to disease controls. Severity Score = 0-7, Mean ± SE. Osteoclast Count (in 5 different joint areas of potential disease related bone resorption). . Statistical analysis was performed by 1-way ANOVA with Dunn's multiple comparisons test. * p<0.001

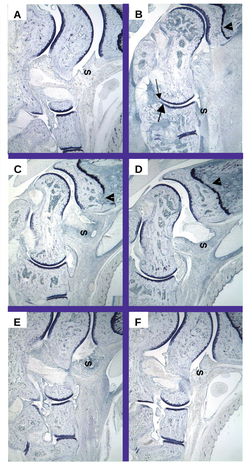
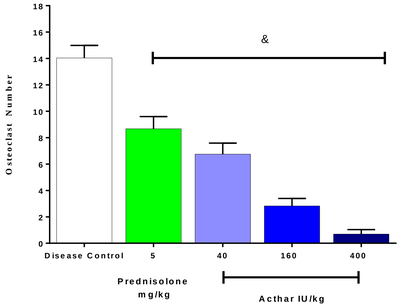


Figure 3. Histopathological images. A) Naive animals displayed no lesions. (**B**) Disease animals have very severe inflammation with minimal cartilage damage and minimal pannus and severe bone resorption. **C**) Disease animals treated with prednisolone showed moderate inflammation, very minimal cartilage damage and pannus, and marked bone resorption. Animals treated with 40 **(D)**, 160 **(E),** or 400 **(F)** IU/kg of RCI show a dose responsive reduction in inflammation and bone resorption. Synovium (S), Pannus (small arrow), cartilage damage (large arrow), bone resorption (arrowhead)

Figure 4. Treatment with RCI decreases the numbers of osteoclast in rats with adjuvant-induced arthritis



Osteoclasts were counted in areas of potential disease related bone resorption (subchondral bone or marginal zones) on 100x magnification using an ocular micrometer. Five fields were counted for each section and the mean for each animal was determined. Osteoclasts in normal bone marrow or at the growth plate were not counted. Values are reported as mean ± SEM (n = 7–8 animals per group). Osteoclast counts were significantly reduced in rats by 52%, 80%, and 95% compared to disease control when treated with RCI at 40, 160, and 400 IU/kg, respectively. Prednisolone reduce osteoclast numbers by 38% compared to the disease control. . Statistical analysis was performed by 1-way ANOVA with a Holm-Sidak post-hoc analysis . $^{\&}P < 0.0001$ significant difference from disease control group.

Conclusion

- **Significant and dose responsive inhibition of disease induction by RCI whereas prednisolone treatment alone only showed a minor benefit.**
- **Histological results suggest response is driven by suppression of joint inflammation and bone resorption**
- **Reduction in osteoclast numbers suggesting a potential bone sparing effect.**
- **These data support the potential efficacy of H.P. Acthar® Gel as an adjunctive therapy for patients with rheumatoid arthritis.**

The study was conducted by Bolder BioPath Inc., Boulder Colorado, and funded by Mallinckrodt Pharmaceuticals, a registered business name of Mallinckrodt plc. Dale Wright, Ben Zweifel, Steve Settle, Rick Fitch are employees of a Mallinckrodt company and stockholders of Mallinckrodt plc.
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Presented at the American College of Rheumatology Annual Meeting • Chicago, IL • October 19-24, 2018.