

Abstract

Background: Mitogen-activated protein kinases (MAPKs) are known to participate in multiple cellular processes. The MAPK/ERK kinase (MEK) signaling pathway is activated during many inflammatory processes. Blockade of this pathway may represent an approach to reducing inflammatory disease. Aim: Assess the anti-inflammatory activity of two potent and highly selective MEK1/2 inhibitors, RDEA436 and RDEA119, in a rat model of arthritis.

Objectives: Assess the anti-inflammatory activity of two potent and highly selective MEK1/2 inhibitors, RDEA436 and RDEA119, in a rat model of arthritis.

Methods: Male Dark Agouti rats were injected intradermally with pristane (2,6,10,14-Tetramethylpentadecane; 150 µl/rat) to induce arthritis. Arthritic rats were then dosed orally with RDEA436 (BID), RDEA119 (QD) or methotrexate (MTX, QD) for 14 consecutive days starting on the day of disease onset. MTX was dosed alone or was combined with one of the two MEK inhibitors. Naïve control received no pristane injection or oral treatment. Vehicle control received pristane injection and vehicle administered orally. Paw and ankle diameter was measured on day 15 of dosing.

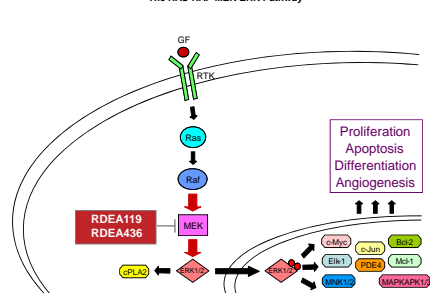
Results: (i) RDEA436 (0.15, 0.5 & 1.5 mg/kg) treatment reduced disease-mediated body weight loss and significantly reduced the ankle inflammation by 44, 49 and 66 % respectively compared to vehicle. MTX (0.05 & 0.15 mg/kg) treatment reduced ankle inflammation by 55 and 31%, respectively. (ii) In a different experiment, RDEA436 (0.1 mg/kg) or MTX (0.05 mg/kg) treatment did not reduce the paw inflammation when dosed as monotherapy; however, the combination of both compounds together reduced paw inflammation by 89% compared to vehicle. (iii) Compared to vehicle, RDEA119 (0.3, 1, 3 mg/kg) treatment significantly reduced body weight loss and significantly reduced the number of inflamed digits by 58, 64 and 77% respectively. MTX (0.025 & 0.05 mg/kg) treatment also significantly reduced the number of inflamed digits by 62 and 68% respectively. Similarly, RDEA119 treatment significantly reduced the ankle inflammation by 27, 36 and 80%. In contrast MTX failed to reduce the ankle inflammation at 0.025 mg/kg but was slightly efficacious at 0.05 mg/kg (12% reduction). (iii) RDEA119 (0.1 mg/kg) combined with MTX (0.05 mg/kg) significantly reduced the number of inflamed digits by 85 % while RDEA119 alone was effective by 44%.

Conclusion: Blockade of the MEK signaling pathway by RDEA436 and RDEA119 is potentially efficacious for the treatment of inflammatory diseases. In addition, combination therapies of RDEA436 or RDEA119 with MTX are promising therapeutic approaches given their differential mechanisms of action, and additive to synergistic activity demonstrated in this arthritis model.

Introduction

The extracellular signal-regulated kinase (ERK) pathway plays a critical role in the control of cellular activation, proliferation, and the production of inflammatory mediators. Suppression of the ERK pathway by inhibitors of MEK-1 and MEK-2 (MEK1/2) represents a potential therapeutic approach for treating chronic inflammatory diseases including rheumatoid arthritis beyond the current therapies (steroids, methotrexate and anti-TNF inhibitors). RDEA119 and RDEA436, novel selective MEK1/2 inhibitors have demonstrated inhibition of ERK phosphorylation (pERK) and cytokine production upon stimulation of human peripheral blood mononuclear cells with a phorbol ester mitogen.

The RAS-RAF-MEK-ERK Pathway

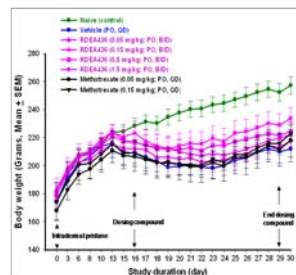


Proliferation
Apoptosis
Differentiation
Angiogenesis

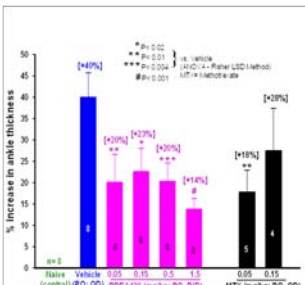
Results

Figure 1. RDEA436 exhibits efficacy in pristane-induced arthritis

(A) Oral RDEA436 reduces body weight loss associated with the development of arthritis

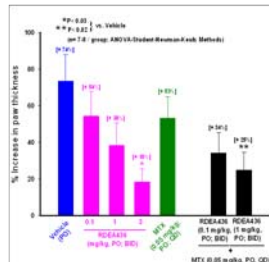


(B) Oral RDEA436 significantly reduces intradermal pristane-induced ankle joint inflammation



Methods: Arthritis was induced by intradermal injection of pristane (150 µl) at the base of tail (day 0) in Dark Agouti rats. RDEA436 (0.05-1.5 mg/kg, PO, BID) was administered for 14 consecutive days starting on the day of disease onset (day 16). Methotrexate (0.05 & 0.15 mg/kg) was used as positive control drug. Paw and ankle joint thickness were measured using a digital caliper.

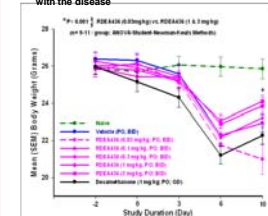
Figure 2. The combination of RDEA436 and methotrexate is efficacious in pristane-induced arthritis



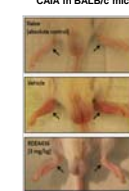
Methods: The 14-day treatment with RDEA436 (0.1-3 mg/kg, PO, BID) or Methotrexate (0.05 mg/kg, PO, QD) started on day 17 post-pristane injection. Arthritis severity was assessed as previously described.

Figure 3. RDEA436 Potently Inhibits Collagen-Antibody Induced Arthritis (CAIA) with an improved side effect profile

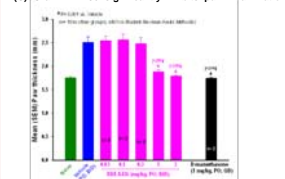
(A) Oral RDEA436 reduces body weight loss associated with the disease



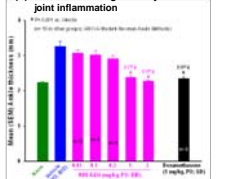
(B) Morphological features of CAIA in BALB/c mice



(C) Oral RDEA436 significantly inhibits paw inflammation



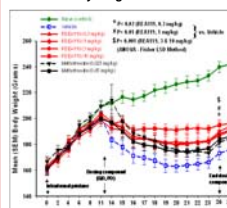
(D) Oral RDEA436 significantly inhibits ankle joint inflammation



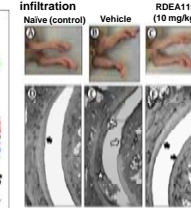
Methods: Male Balb/c mice were injected IV (tail vein) with 2 mg of collagen antibody cocktail (Chondrex) on day 0. RDEA436 (0.03 - 3 mg/kg, PO, BID) or dexamethasone (1 mg/kg, PO, QD) were administered continuously from day -2 until study termination. LPS (50 µg) was injected IP on day 3 post-collagen antibody administration. Arthritis severity was assessed on day 10.

Figure 4. Oral RDEA119 inhibits joint inflammation and prevents cartilage damage in pristane-induced arthritis

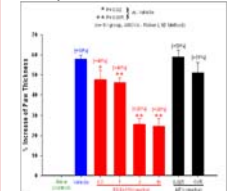
(A) RDEA119 reduces pristane-induced body weight loss



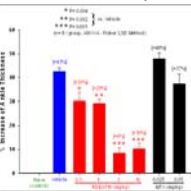
(B) Oral RDEA119 prevents cartilage damage and tissue infiltration



(C) Oral RDEA119 reduces paw inflammation as index of disease severity



(D) Oral RDEA119 dose dependently reduces ankle joint inflammation as index of disease severity



Methods: RDEA119 (0.3-10 mg/kg, PO, QD) or methotrexate (0.025 & 0.05 mg/kg, PO, QD) were administered for 14 consecutive days starting on day 12 post-pristane injection (disease onset) in Dark agouti rats. Arthritis severity was assessed as previously described.

Conclusions

- RDEA436 (0.1, 1 & 3 mg/kg) displayed a dose-dependent reduction of pristane-induced arthritis in Dark Agouti rats
- RDEA436 (1 mg/kg) combined with methotrexate (0.05 mg/kg) displayed enhanced efficacy in vivo.
- RDEA119 suppressed pristane-induced arthritis with better efficacy than methotrexate
- These data support the continued development of MEK inhibitors for treatment of inflammatory diseases including arthritis existing the possibility of combination therapy with low dose(s) of existing disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate.