

Combination benefit of PEGylated soluble tumor necrosis factor receptor type I (PEG sTNF-RI) and dexamethasone or indomethacin in adjuvant arthritic rats

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Abstract. *Objective:* To determine the potential combination benefit receptor of treatment with PEGylated soluble tumor necrosis factor type I (PEG sTNF-RI) and dexamethasone (dex) or indomethacin (indo) in adjuvant arthritic rats.

Subjects: 160 male Lewis Rats.

Treatment: PEG sTNF-RI, dex, indo.

Methods: Rats with adjuvant arthritis were given daily oral dex (0.025 or 0.006 mg/kg) or indo (0.5 or 0.25 mg/kg) day 9–14, alone or in combination with PEG sTNF-RI (sc on days 9, 11, and 13 of arthritis). Efficacy was monitored by volume measurement of ankle joints, final paw weights and histologic evaluation with particular emphasis on bone lesions.

Results: Treatment with 1 mg/kg PEG sTNF-RI alone resulted in 27% inhibition of final paw weights, dex alone (0.025 mg/kg) gave 25% inhibition and the combination resulted in 58% inhibition. Histologic evaluation of ankle joints demonstrated 48% inhibition of bone resorption with PEG sTNF-RI alone, 55% inhibition with dex alone and the combination treatment inhibited bone resorption by 100%. Inactive doses of PEG sTNF-RI (0.3 mg/kg) and dex (0.006 mg/kg) when combined resulted in 39% inhibition of paw swelling (AUC) and 39% inhibition of bone resorption. Combination treatment with indomethacin resulted in slight additive effects on inflammation parameters but no additive effects on bone resorption.

Conclusion: Combination therapy with PEG sTNF-RI and dexamethasone results in additive or synergistic effects depending on the dose. Combination therapy with indomethacin resulted in slight additive effects on paw swelling parameters, but no additive benefit on bone resorption. Data from these studies support the clinical investigation of the use of combination therapy of PEG sTNF-RI and dex or other corticosteroids in rheumatoid arthritis patients.

Key words: Tumor necrosis factor – Soluble receptor – Adjuvant arthritis – Dexamethasone – Indomethacin

Introduction

Rheumatoid arthritis (RA) is a chronic disease characterized by inflammation of the joints with concomitant destruction of cartilage and bone. The involvement of cytokines, particularly IL-1 and TNF- α in the pathogenesis of RA is now well accepted as a result of numerous studies in animal models as well as in patients with the disease [1–4]. Soluble TNF receptors and antibodies to TNF have been shown to be clinically efficacious in rheumatoid arthritis patients [5–9]. Animal models of arthritis in which these agents were evaluated, predicted the excellent human clinical response [10–17].

Dexamethasone is used in low doses as a therapeutic agent in the treatment of RA. Various toxicities associated with chronic administration of corticosteroids preclude their chronic use at highly effective anti-inflammatory or immunomodulatory doses [18, 19]. Similarly, nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin are used in the treatment of RA but are generally thought to be symptomatic therapy rather than disease modifying. Gastrointestinal and renal toxicities preclude their use at high doses for prolonged periods [20].

Rat adjuvant arthritis is an experimental model of polyarthritis which has been widely used for preclinical testing of numerous anti-arthritic agents which are either undergoing preclinical or clinical investigation or are currently used as therapeutics in this disease. The hallmarks of this model are reliable onset of robust polyarticular inflammation, marked bone resorption and periosteal bone proliferation [21–23]. Cartilage destruction occurs but is disproportionately mild in comparison to the inflammation and bone destruction that

occurs. Corticosteroids such as dex, indo and TNF inhibitors such as PEG sTNF-RI have been shown to be efficacious in this model [8, 24].

In the present study, we evaluated the efficacy of PEG sTNF-RI alone and in combination with ineffective and effective doses of dex on inflammation associated paw swelling and bone resorption in adjuvant arthritic rats in an effort to determine potential additive or synergistic effects of the combination therapy. In similar studies, effective and minimally effective doses of indo were evaluated alone and in combination with PEG sTNF-RI.

Materials and methods

Animals

Male Lewis rats (200–250 g, Charles River, Portage, MI, USA) were used in these studies. Animals were allowed to acclimate for at least 3 days prior to initiation of experimentation. Rats were housed 4/cage in polycarbonate cages and were allowed ad libitum access to food and water. All animal use was in accordance with USDA guidelines for humane care and was approved by an internal animal care and use committee.

Materials

Recombinant PEG sTNF-RI and its vehicle were produced at Amgen (Boulder, CO, USA). PEG sTNF-RI is a recombinant *E. coli* form of the 'high-affinity' p55 soluble tumor necrosis factor receptor type I (sTNF-RI) to which a 30 kd polyethylene glycol (PEG) molecule is attached (25–28). Dex and indo (prepared in 1% carboxymethylcellulose, dose volume = 5 ml/kg) were purchased from Sigma (St. Louis, MO, USA). Freund's complete adjuvant (FCA) was obtained from Sigma. The synthetic adjuvant N, N-dioctyldecyl-N', N'-bis(2-hydroxyethyl) propanediamine (LA) was obtained from BolderPATH Inc. (Boulder, CO, USA).

Induction of adjuvant arthritis

Male rats were given single sc injections (base of tail) of 100 µl of FCA to which 5 mg of LA was added. Treatments were initiated on day 9 (approximate onset of paw inflammation) and continued daily through day 14 for dex and indo. PEG sTNF-RI sc treatments were given on days 9, 11, and 13 of arthritis. Arthritis onset occurred on day 9 or 10 post-adjuvant injection.

Clinical assessment of adjuvant arthritis

Volume measurements of both hind paws were done prior to the onset of arthritis, and then every other day until the study was terminated on day 15 post-injection of the adjuvant. At termination, the tibiotarsal joint was transected at the level of the medial and lateral malleolus for determination of paw weights as another measure of inflammation. Paws were then collected into formalin for histopathologic evaluation. Body weights were also determined.

Histopathology

Ankle joints were collected into 10% neutral buffered formalin for at least 24 hours prior to placement in Surgipath decalcifier I (Grayslake, IL, USA) for approximately 1 week. When decalcification was com-

plete, the digits were trimmed and the ankle joint was transected in the longitudinal plane to give approximately equal halves. These were processed for paraffin embedding, sectioned and stained with hematoxylin and eosin. Multiple sections were prepared to ensure that the distal tibia was present with both cortices and that abundant distal tibial medullary space was available for evaluation. Adjuvant arthritic ankles were given scores of 0–5 for bone resorption and inflammation according to the following criteria:

Bone resorption

0 = normal; 1 = minimal = small areas of resorption in distal tibial trabecular or cortical bone, not readily apparent on low magnification, rare osteoclasts; 2 = mild = more numerous areas of resorption in distal tibial trabecular or cortical bone, apparent on low magnification, osteoclasts more numerous; 3 = moderate = obvious resorption of medullary trabecular and cortical bone without bilateral full thickness defects in cortex, loss of some medullary trabeculae, lesion apparent on low magnification, osteoclasts more numerous; 4 = marked = full thickness bilateral defects in cortical bone, often with distortion of profile of remaining cortical surface, marked loss of medullary bone of distal tibia, numerous osteoclasts, no resorption in smaller tarsal bones; 5 = severe = full thickness bilateral defects in cortical bone, often with distortion of profile of remaining cortical surface, marked loss of medullary bone of distal tibia, numerous osteoclasts, resorption also present in smaller tarsal bones.

Inflammation

0 = normal; 1 = minimal infiltration of inflammatory cells in periarticular tissue; 2 = mild infiltration with mild edema; 3 = moderate infiltration with moderate edema; 4 = marked infiltration with marked edema; 5 = severe infiltration with severe edema.

Cartilage damage was not scored in the adjuvant model because we have generally found this to be a minor feature of the lesion and therefore not reliable for evaluation of potential treatment effects.

Study design

All test groups except normal controls (n = 4) contained 7 rats. Treatment with PEG sTNF-RI was by sc injection on days 9, 11 and 13 post-adjuvant injection. Oral dex doses were 0.025 or 0.006 mg/kg beginning on day 9 and continuing through day 14. Oral indo doses were 0.5 or 0.25 mg/kg beginning on day 9 and continuing through day 14. All rats in this study were treated similarly in that all were given oral doses of vehicle or drug and all were given sc injections of vehicle or PEG sTNF-RI. Rats were terminated on day 15 for determination of final paw and body weights.

Statistical analysis

Clinical data for paw volume were analyzed by determining the area under the dosing curve with subsequent analysis of variance. For calculation of AUC, the daily volume of ankle joints (using a water displacement system) for each rat was entered and plotted with the Statistical Analysis Software (SAS, Cary, NC, USA) where the area between the treatment days after the onset of disease to the termination day was computed. Means for each group were determined and % inhibition from arthritis controls was calculated by comparing values for treated and normal animals. ANOVA was performed to test for differences among the treatment groups. If the ANOVA was significant ($p < 0.05$), pairwise Student's t-tests were performed to test for differences between the treatments and the arthritis control, and between the individual treatments and combination therapy. A similar nonparametric analysis was performed for bone resorption and inflammation using Kruskal-

Wallis tests and pairwise Wilcoxon tests. Pairwise differences were deemed significant if $p \leq 0.05$.

Paw weights (mean \pm SE) for each group were analyzed for differences using the Student's t-Test. In both cases, significance was set at $p \leq 0.05$.

Percent inhibition of paw volume and AUC was calculated using the following formula:

$$\% \text{ inhibition} = A - B/A \times 100$$

A = mean disease control - mean normal

B = mean treated - mean normal

Results

Combination therapy with PEG sTNF-RI and dexamethasone

Treatment of adjuvant arthritic rats with 1 mg/kg PEG sTNF-RI resulted in 23% inhibition of AUC for paw swelling and 27% inhibition of final paw weight (Fig. 1A, B). There were no beneficial effects of either treatment on final body weight (data not shown). Treatment of adjuvant arthritic rats with daily oral doses of dex (0.025 mg/kg) on days 9–14, resulted in 27% inhibition of AUC for paw swelling, 25% inhibition of final paw weight and no inhibition of final body weight change (Fig. 1A, B). Concurrent treatment with PEG sTNF-RI and dex at these moderately active doses generally resulted in additive benefit. The combination treatment resulted in 70% inhibition of AUC for paw swelling and 58% inhibition of final paw weight (Fig. 1A, B). There was no combination benefit on adjuvant disease induced final body weight effects (data not shown). Histologic evaluation of ankle joints from rats treated with PEG sTNF-RI at 1 mg/kg demonstrated 31% inhibition of inflammation and 48% inhibition of bone resorption (Fig. 1C). Treatment with dex resulted in 36% inhibition of inflammation and 55% inhibition of bone resorption. The combination of 1 mg/kg PEG sTNF-RI and 0.025 mg/kg dex resulted in 100% inhibition of bone resorption and 64% inhibition of inflammation, both significantly better than either treatment alone (Fig. 1C).

Treatment of adjuvant arthritic rats with 0.3 mg/kg PEG sTNF-RI resulted in no significant inhibition of AUC for paw swelling or final paw weight (Fig. 2A, B). There were no beneficial effects of treatment on final body weight (data not shown). Treatment of adjuvant arthritic rats with daily oral doses of dex (0.006 mg/kg) on days 9–14, resulted in no inhibition of AUC for paw swelling, final paw weight, or final body weight change (Fig. 2A, B). Concurrent treatment with

PEG sTNF-RI and dex at these inactive doses resulted in benefit on some parameters. The combination treatment of 0.3 mg/kg PEG sTNF-RI and 0.006 mg/kg dex resulted in 39% inhibition of AUC for paw swelling and 17% inhibition (not significant) of final paw weight (Fig. 2A, B). Combination benefit was not seen on adjuvant disease induced body

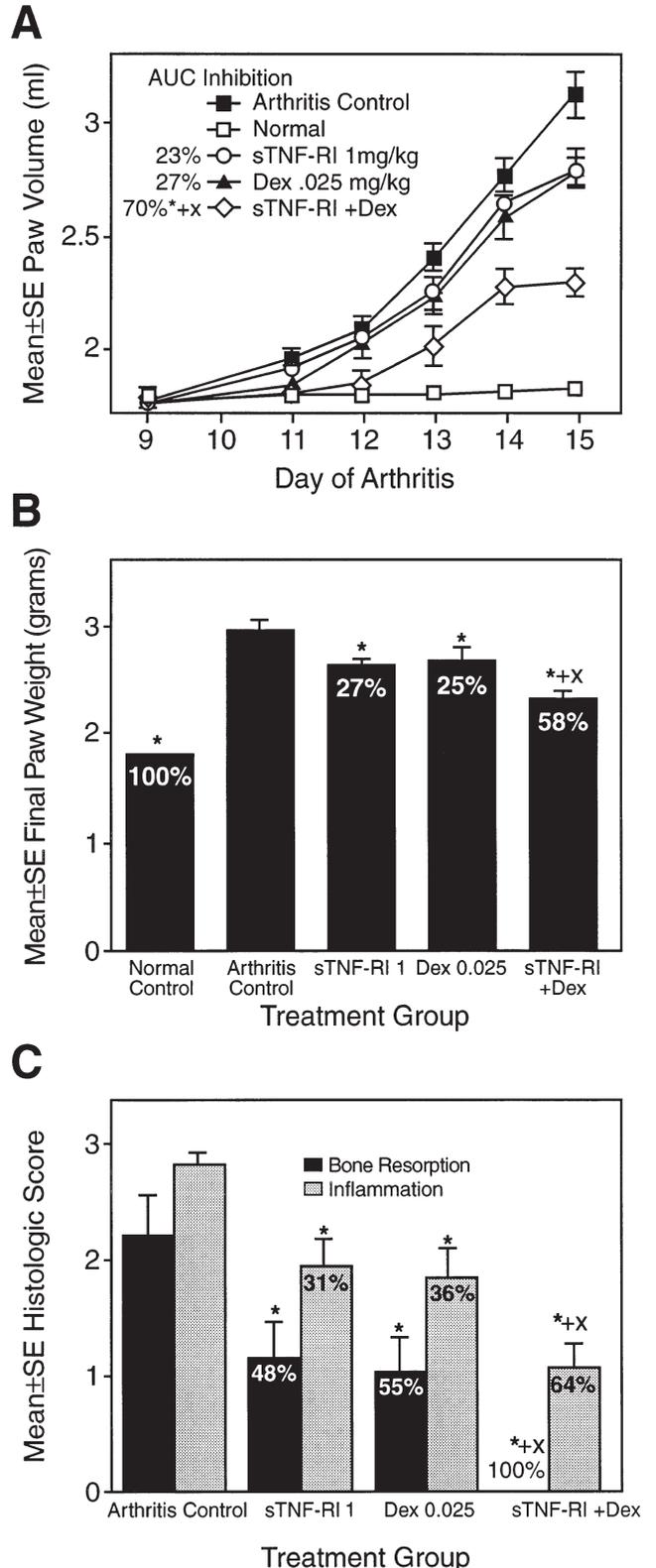
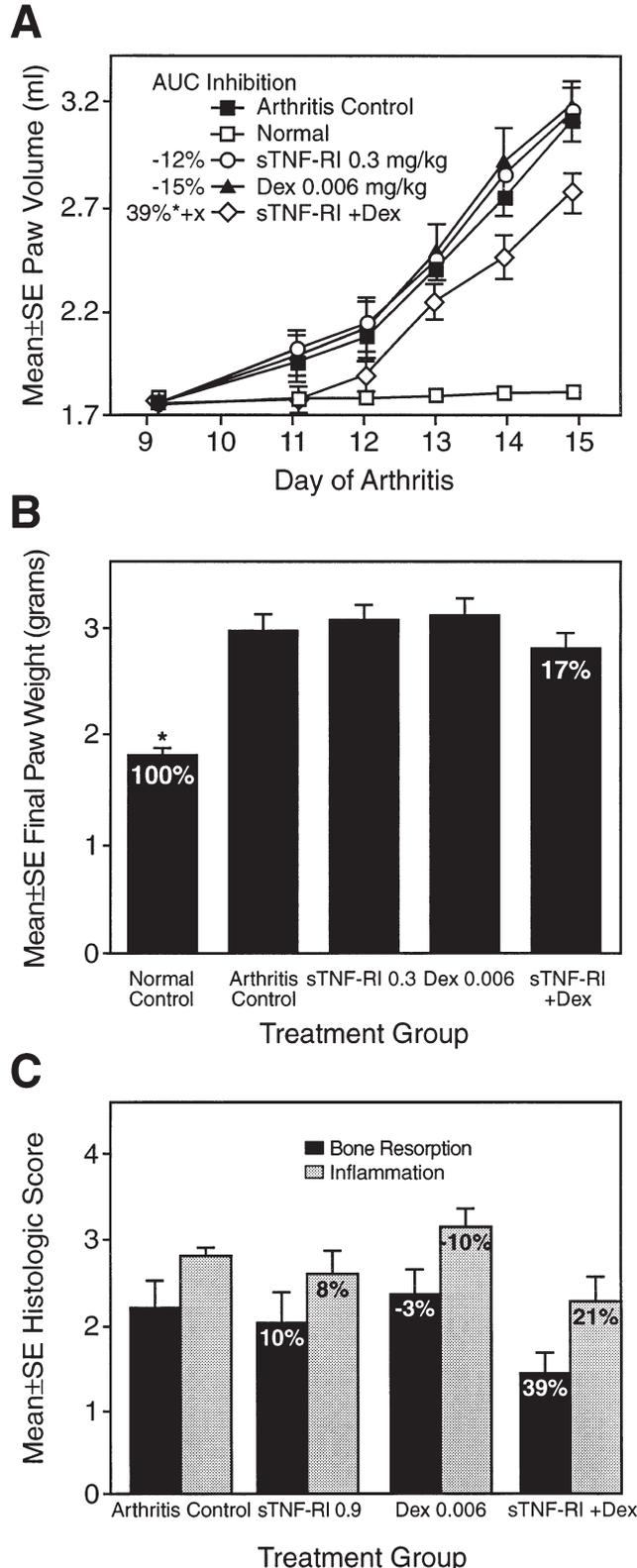


Fig. 1. Paw volume over time (A) in adjuvant arthritic rats treated with 1 mg/kg PEG sTNF-RI sc, on days 9, 11 and 13 of arthritis, dexamethasone po (0.025 mg/kg) on days 9–14, or the combination. AUC % inhibition (calculated from raw data) is shown to the left of legend. * $p < 0.05$, ANOVA compared to arthritis control, + $p < 0.05$, ANOVA compared to PEG sTNF-RI or $\times p < 0.05$ to dex treatment. (B) Final paw weights from rats treated with PEG sTNF-RI, dexamethasone or the combination. Percent inhibition from arthritis control is shown on the bars. * $p < 0.05$, 2 tailed t-test to arthritis control, + $p < 0.05$, two-tailed t-test to PEG sTNF-RI or $\times p < 0.05$ to dex treatment. (C) Histologic inflammation and bone resorption scores * $p < 0.05$, Kruskal-Wallis test to arthritis control, + $p < 0.05$ to PEG sTNF-RI and $\times p < 0.05$ to dex treatment. % on bars = % inhibition from arthritis control.

weight effects (data not shown). Histologic evaluation of sections from rats treated with inactive doses of PEG sTNF-RI (0.3 mg/kg) revealed 10% inhibition of bone resorption and 8% inhibition of inflammation, both not significantly different from arthritis control values (Fig. 2C). Similarly,

treatment with 0.006 mg/kg dex did not show any benefit on these histologic parameters. The combination treatment resulted in 39% inhibition of bone resorption and 21% inhibition of inflammation (neither significantly different from arthritis controls or from each individual treatment (Fig. 2C).

The potential for combination benefit of an inactive dose of PEG sTNF-RI (0.3 mg/kg) and an active dose of dex (0.025 mg/kg) was also investigated. This combination resulted in synergistic effects on both paw swelling parameters and bone resorption (Fig. 3A, B, C).



Combination therapy with PEG sTNF-RI and indomethacin

Clinical parameters. Treatment of adjuvant arthritic rats with 1 mg/kg PEG sTNF-RI resulted in 23% inhibition of AUC for paw swelling and 36% inhibition of final paw weight (Fig. 4A, B). There were no beneficial effects of treatment on final body weight (data not shown). Treatment of adjuvant arthritic rats with daily oral doses of indo (0.5 mg/kg) on days 9–14, resulted in 52% inhibition of AUC for paw swelling, 61% inhibition of final paw weight, no inhibition of body weight change (data not shown). Concurrent treatment with PEG sTNF-RI and indo at this moderately active dose of TNF-RI and close to maximally active dose of indo resulted in slight additive benefit on paw swelling parameters. The combination treatment of 1 mg/kg PEG sTNF-RI and 0.5 mg/kg indo resulted in 66% inhibition of AUC for paw swelling and 69% inhibition of final paw weight (Fig. 4A). There was no combination benefit on adjuvant disease induced body weight effects (data not shown). Histologic evaluation of ankle joints from rats treated with PEG sTNF-RI at 1 mg/kg demonstrated 14% inhibition of inflammation and 17% inhibition of bone resorption (Fig. 3C). Treatment with indo (0.5 mg/kg) resulted in 38% inhibition of inflammation and 33% inhibition of bone resorption. The combination of 1 mg/kg PEG sTNF-RI and 0.5 mg/kg indo resulted in 39% inhibition of bone resorption and 50% inhibition of inflammation (Fig. 4C).

In a different study, treatment of adjuvant arthritic rats with 1 mg/kg PEG sTNF-RI resulted in 45% inhibition of AUC for paw swelling and 37% inhibition of final paw weight (Fig. 5A, B). Treatment of adjuvant arthritic rats with daily oral doses of indo (0.25 mg/kg) on days 9–14, resulted in 17% inhibition of AUC for paw swelling, and 42% inhibition of final paw weight. Concurrent treatment with PEG sTNF-RI and indo at these moderately active doses resulted

Fig. 2. Paw volume over time (A) in adjuvant arthritic rats treated with 0.3 mg/kg PEG sTNF-RI sc, on days 9, 11 and 13 of arthritis, dexamethasone po (0.006 mg/kg) on days 9–14, or the combination. AUC % inhibition (calculated from raw data) is shown to the left of the legend. * $p < 0.05$, ANOVA compared to arthritis control, + $p < 0.05$, ANOVA compared to PEG sTNF-RI or * $p < 0.05$ to dex treatment. (B) Final paw weights from rats treated with PEG sTNF-RI, dexamethasone or the combination. Percent inhibition from arthritis control is shown on the bars. * $p < 0.05$, two-tailed t-test to arthritis control, + $p < 0.05$, two-tailed t-test to PEG sTNF-RI or * $p < 0.05$ to dex treatment. (C) Histologic inflammation and bone resorption scores * $p < 0.05$, Kruskal-Wallis test to arthritis control, + $p < 0.05$ to PEG sTNF-RI and * $p < 0.05$ to dex treatment. % on bars = % inhibition from arthritis control.

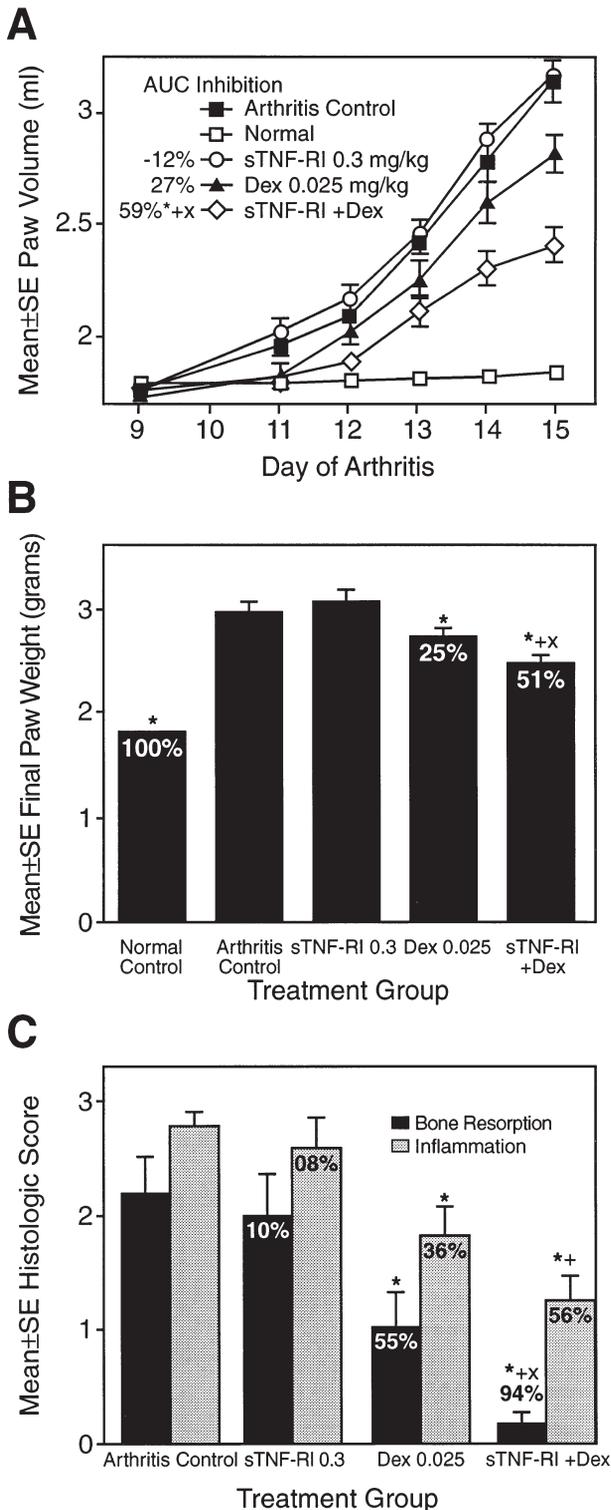


Fig. 3. Paw volume over time (A) in adjuvant arthritic rats treated with 0.3 mg/kg PEG sTNF-RI sc, on days 9, 11 and 13 of arthritis, dexamethasone po (0.025 mg/kg) on days 9–14, or the combination. AUC % inhibition (calculated from raw data) is shown to the left of legend. *p < 0.05, ANOVA compared to arthritis control, +p < 0.05, ANOVA compared to PEG sTNF-RI or *p < 0.05 to dex treatment. (B) Final paw weights from rats treated with PEG sTNF-RI, dexamethasone or the combination. Percent inhibition from arthritis control is shown on the bars. *p < 0.05, two-tailed t-test to arthritis control, +p < 0.05, two-tailed t-test to PEG sTNF-RI or *p < 0.05 to dex treatment. (C) Histologic inflammation and bone resorption scores *p < 0.05, Kruskal-Wallis test to arthritis control, +p < 0.05 to PEG sTNF-RI and *p < 0.05 to dex treatment. % on bars = % inhibition from arthritis control.

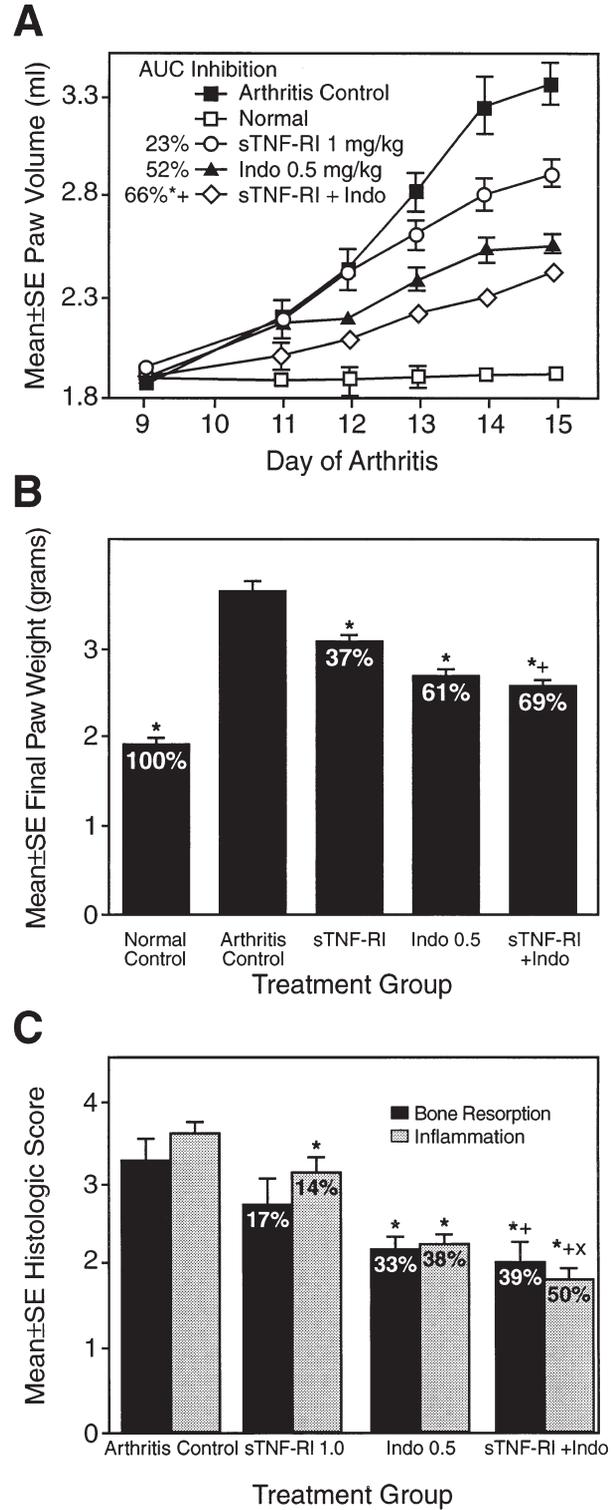
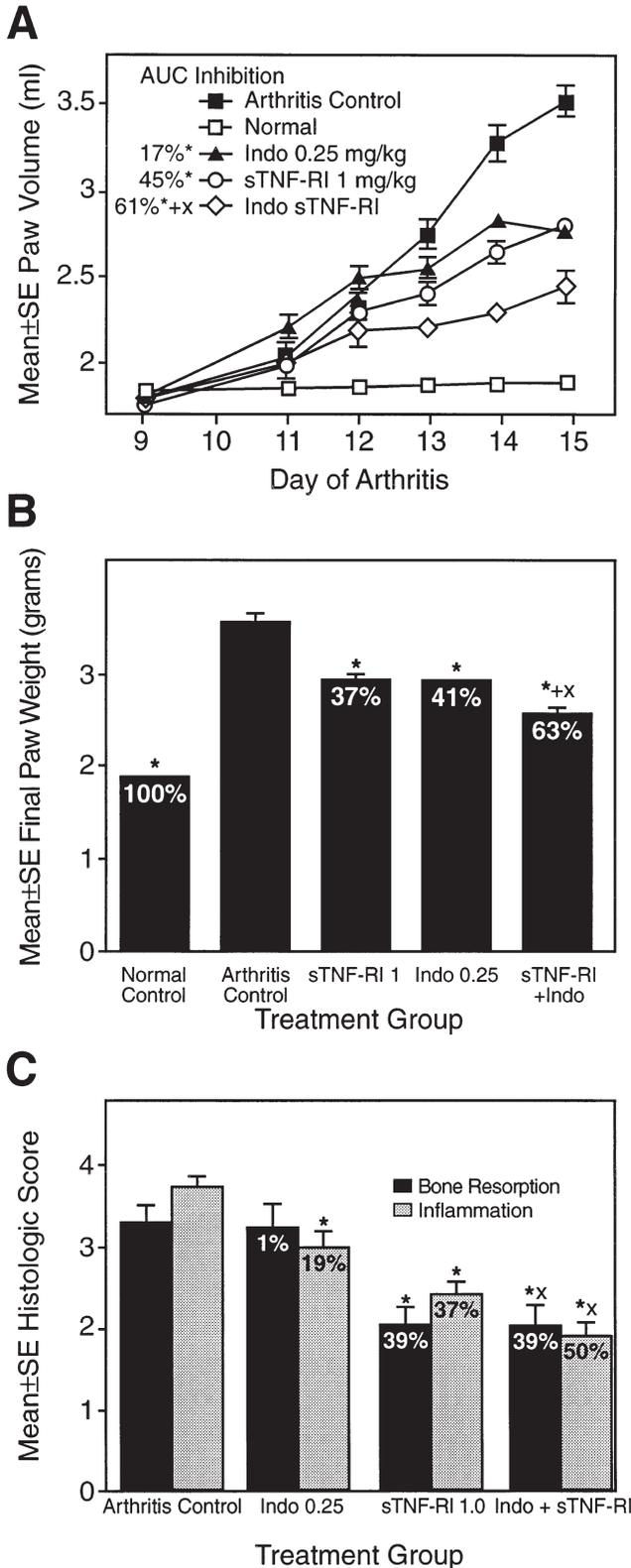


Fig. 4. Paw volume over time (A) in adjuvant arthritic rats treated with 1 mg/kg PEG sTNF-RI sc, on days 9, 11 and 13 of arthritis, indomethacin po (0.5 mg/kg) on days 9–14, or the combination. AUC % inhibition (calculated from raw data) is shown to the left of legend. *p < 0.05, ANOVA compared to arthritis control, +p < 0.05, ANOVA compared to PEG sTNF-RI or *p < 0.05 to dex treatment. (B) Final paw weights from rats treated with PEG sTNF-RI, dexamethasone or the combination. Percent inhibition from arthritis control is shown on the bars. *p < 0.05, two-tailed t-test to arthritis control, +p < 0.05, two-tailed t-test to PEG sTNF-RI or *p < 0.05 to dex treatment. (C) Histologic inflammation and bone resorption scores *p < 0.05, Kruskal-Wallis test to arthritis control, +p < 0.05 to PEG sTNF-RI and *p < 0.05 to dex treatment. % on bars = % inhibition from arthritis control.

in combination benefit on paw swelling parameters. The combination treatment of 1 mg/kg PEG sTNF-RI and 0.25 mg/kg indo resulted in 61% inhibition of AUC for paw swelling and 63% inhibition of final paw weight (Fig. 5A, B). Combination benefit was not seen on adjuvant disease in-

duced body weight effects (data not shown). Histologic evaluation of sections from rats treated with PEG sTNF-RI (1 mg/kg) revealed 39% inhibition of bone resorption and 37% inhibition of inflammation, (Fig. 5C). Treatment with 0.25 mg/kg indo did not show any benefit on inhibition of bone resorption and only modest (19%) inhibition of inflammation. The combination treatment resulted in 39% inhibition of bone resorption and 50% inhibition of inflammation and therefore reflected the activity of PEG sTNF-RI on the bone resorption parameter, with a slight additive benefit on the inflammation parameter (Fig. 5C).



Discussion

In this series of studies, various doses of PEG sTNF-RI and dex or indo, alone and in combination were given to adjuvant arthritic rats in an effort to determine potential for combination benefit. Treatment of adjuvant arthritic rats with 1 mg/kg PEG sTNF-RI on days 9, 11 and 13 of arthritis resulted in beneficial effects on soft tissue swelling (23–45% inhibition of AUC, 27–37% inhibition of final paw weights) and bone resorption (17–48% inhibition) in the 3 studies in which it was currently evaluated. Treatment of adjuvant arthritic rats with a dose of 0.3 mg/kg did not result in significant inhibition of inflammation or bone resorption in this study whereas in a previous study, a modest (approximately 25% inhibition) was seen on both parameters [8]. Therefore, the activity of PEG sTNF-RI in the currently reported studies is reasonably consistent with our previously reported data [8, 28] and reflects the range of variability seen in this model. Higher doses (3 mg/kg) of PEG sTNF-RI have resulted in 50–90% inhibition of inflammation and bone resorption (respectively) parameters [28]. This higher dose was not used in the current combination studies since it has the potential to be extremely effective alone, therefore making demonstration of combination benefit difficult, especially on the important parameter of bone resorption.

Low dose dex has previously been shown to be effective in suppressing paw inflammation and bone resorption in adjuvant arthritic rats [24] and in low doses, corticosteroids are an effective clinical treatment for rheumatoid arthritis [18, 19]. In our studies, we have found that deleterious effects on body weight and other parameters occur at the efficacious (ED₅₀) doses of dex [24]. Potential for toxicity also precludes dosing at high levels in the clinic. Therefore, the hypothesis is that low doses of corticosteroids such as dex in combina-

Fig. 5. Paw volume over time (A) in adjuvant arthritic rats treated with 1 mg/kg PEG sTNF-RI sc, on days 9, 11 and 13 of arthritis, indomethacin po (0.25 mg/kg) on days 9–14, or the combination. AUC % inhibition (calculated from raw data) is shown to the left of legend. * p < 0.05, ANOVA compared to arthritis control, + p < 0.05, ANOVA compared to PEG sTNF-RI or x p < 0.05 to dex treatment. (B) Final paw weights from rats treated with PEG sTNF-RI, dexamethasone or the combination. Percent inhibition from arthritis control is shown on the bars. * p < 0.05, two tailed t-test to arthritis control, + p < 0.05, two-tailed t-test to PEG sTNF-RI or x p < 0.05 to dex treatment. (C) Histologic inflammation and bone resorption scores * p < 0.05, Kruskal-Wallis test to arthritis control, + p < 0.05 to PEG sTNF-RI and x p < 0.05 to dex treatment. % on bars = % inhibition from arthritis control.

tion with other agents (preferably nontoxic ones like PEG sTNF-RI) might provide additive and potentially synergistic benefit and hence have potential for better disease modification with less risk of deleterious effects. Numerous studies in different laboratories have shown that corticosteroids such as dex inhibit TNF production in various animal models of inflammation [29]. Therefore, it is reasonable to assume that the combination of these 2 agents would potentially result in at least additive effects. In our combination studies, we tested a moderately active (0.025 mg/kg) and an inactive (0.006 mg/kg) dose of dex alone and in combination with a moderately active (1 mg/kg) and marginally active to inactive (0.3 mg/kg) dose of PEG sTNF-RI. Our results show additive benefit on both paw swelling and histologic parameters of bone resorption and inflammation when an effective dose of dex (0.025 mg/kg) and an effective dose of PEG sTNF-RI (1 mg/kg) are used in combination. In this study, these combined agents resulted in 100% inhibition of bone resorption. The combination of an inactive dose of PEG sTNF-RI (0.3 mg/kg) and an active (0.025 mg/kg) dose of dex also results in combination benefit which in this case is synergistic. Interestingly, when inactive doses of both agents (0.006 mg/kg-Dex and 0.3 mg/kg PEG sTNF-RI) were combined, 39% inhibition of paw swelling AUC was achieved along with 39% inhibition (not significant) of bone resorption. These results suggest the potential for combination benefit of low dose corticosteroid treatment with anti-TNF therapies in the clinical setting.

The potential for combination benefit with PEG sTNF-RI and indo was also investigated. NSAIDs such as indomethacin are used clinically in the treatment of RA, but gastrointestinal and renal toxicities preclude dosing at high levels for prolonged periods. Our hypothesis here was similar to what we described for combination therapy with corticosteroids in that it seemed possible that low doses of the potentially toxic NSAIDs in combination with PEG sTNF-RI might provide additive or synergistic benefit thus allowing the use of lower doses of the NSAIDs. Results of our studies showed a modest combination benefit on AUC for paw swelling at both a maximally effective (0.5 mg/kg) and minimally effective (0.25 mg/kg) dose of indo. The effect of the combination on the bone resorption parameter reflected the effect of the most active agent. Therefore, in 2 different testing situations, we were unable to demonstrate combination benefit on this important parameter.

Overall results of these studies support the investigation of combination therapy with low dose corticosteroids such as dex with TNF inhibitors such as PEG sTNF-RI in patients with RA. Our results also suggest that while some additive symptomatic benefit on inflammation parameters may be seen with combinations of NSAIDs and TNF inhibitors, we found no evidence of potential for enhanced inhibition of bone tissue destruction.

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