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Comparative efficacy of a secretory phospholipase A₂ inhibitor with conventional anti-inflammatory agents in a rat model of antigen-induced arthritis

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Abstract

Introduction: Previously, secretory phospholipase A₂ (sPLA₂) inhibition has been used as an adjunct to conventional rheumatoid arthritis therapy in human clinical trials without significant improvement of arthritic pathology. In this study, we compared the efficacy of a potent and orally active group IIa secretory phospholipase A₂ inhibitor (sPLA₂I) to conventional anti-arthritic agents; infliximab, leflunomide and prednisolone, in a rat model of antigen-induced arthritis.

Methods: Initially, to establish efficacy and dose-response, rats were orally dosed with the sPLA₂I (1 and 5 mg/kg) two days prior to arthritis induction, and then daily throughout the 14-day study period. In the second trial, rats were orally dosed with the sPLA₂I (5 and 10 mg/kg/day) beginning two days after the induction of arthritis, at the peak of joint swelling. Separate groups of rats were also dosed with the tumour necrosis factor-alpha (TNF- α) inhibitor infliximab (single 3 mg/kg i.v. injection), leflunomide (10 mg/kg/day, oral) or prednisolone (1 mg/kg/day, oral) at this same time point and used as comparative treatments.

Results: In the pathology prevention trial, both 1 and 5 mg/kg dose groups of sPLA₂I demonstrated a significant reduction in joint swelling and gait disturbances; however, only the higher 5 mg/kg dose resulted in significantly reduced histopathology scores. In the post-induction trial, rats dosed with sPLA₂I showed a significant improvement in joint swelling and gait scoring, whereas none of the conventional therapeutics achieved a significant decrease in both of these two disease markers. Histopathological scoring at the end-point of the study demonstrated significantly reduced median scores in rats treated with 10 mg/kg sPLA₂I and leflunomide.

Conclusions: The results from this study suggest a pathogenic role for sPLA₂ enzymes in this model of arthritis in rats, and the potential clinical utility of sPLA₂ inhibition as a safer, and more effective, alternative to conventional anti-arthritic therapeutics.

Introduction

Rheumatoid arthritis (RA) is an immune-based chronic inflammatory synovitis presenting with pain, stiffness and swelling of the affected joints. RA results in secondary bone and cartilage destruction causing joint deformity. Current therapies include conventional non-steroidal anti-inflammatory agents (NSAIDs), corticosteroids such as prednisolone, disease-modifying anti-rheumatic-drugs,

such as methotrexate or leflunomide, and biological therapies such as the inhibitors of tumour necrosis factor alpha (TNF α), etanercept, adalimumab and infliximab [1]. No single agent is completely effective at treating disease pathology and is devoid of side effects; consequently, a safe and effective treatment for RA remains elusive. In the mid-1980's, phospholipase A₂ (PLA₂) enzymes were found to be highly expressed in the synovial fluid of RA patients [2]. PLA₂ forms a group of enzymes that metabolise phosphoglycerides to release lipid mediators such as lysophospholipids and arachidonic acid. These

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metabolites can be converted into the pro-inflammatory platelet activating factor (PAF) and eicosanoids (prostaglandins, thromboxanes, and leukotrienes), respectively [3]. As opposed to cytosolic PLA₂ enzymes which have physiological functions within virtually all cells [4], secretory PLA₂ (sPLA₂) enzymes are known to be active during inflammation, and thus have been an attractive target for anti-inflammatory drug development [3]. sPLA₂ enzymes also have agonistic activity at the M-type receptor, through which they can promote inflammation via degranulation of mast cells, cytokine release or secretion of elastase, an activator of the complement cascade extrinsic pathway [5-8]. sPLA₂ enzyme concentrations have been found to be elevated in the synovial fluid of patients with RA [2,9]. Correlations have also been found between serum levels of sPLA₂ and clinical markers of disease such as the number of active and effused joints, erythrocyte sedimentation rate, Lansbury index, elevated platelet count, and low hemoglobin in RA patients [10,11]. Arthritic joints have also been shown to have high expression of sPLA₂ group IIa within the synovial lining, while sPLA₂ IIa expression in healthy joints is virtually absent [12]. Furthermore, intra-articular injections of human recombinant sPLA₂ caused acute inflammatory arthritic-like symptoms in rats [13] and rabbits [14], although transgenic mice over-expressing human sPLA₂ did not spontaneously develop arthritis [15,16].

Researchers from Eli Lilly performed a phase I clinical trial using an inhibitor of sPLA₂ group IIa (LY315920) given intravenously to patients with active RA, which provided significant improvement in swollen and tender joints after three days [17]. Following this, a larger scale Phase II trial was conducted to evaluate the oral efficacy of LY333013, a methyl ester prodrug of LY315920. The results from this trial indicated that although there were significant dose-response related improvements after one week of treatment, there was no significant effect following four and eight weeks of treatment [17]. Potential explanations for this failure include the lack of sufficient inhibitor concentration in the synovial fluid to inhibit local joint sPLA₂, and that all patients were already receiving disease-modifying anti-arthritic drug therapy throughout the trial [17,18]. Therefore, there is still a need to establish whether there may be a pathogenic role of sPLA₂ enzymes in RA.

We have previously reported that a synthetic small molecule inhibitor of group IIa sPLA₂ (sPLA₂I; 5-(4-benzyloxyphenyl)-4S-(7-phenylheptanoylamino)-pentanoic acid) is orally active and has therapeutic efficacy in rat models of intestinal ischemia-reperfusion injury [19] and inflammatory bowel disease [20]. There has also been evidence of efficacy with this compound in a small, preliminary investigation in adjuvant-induced arthritic rats [21]. To evaluate this finding, the present study

reports a full investigation of the potential of this agent to prevent and reverse signs of inflammatory disease in the rat antigen-induced arthritis model. In addition, we compared the *in vivo* activity of this sPLA₂I to the conventional anti-arthritic agents, infliximab, leflunomide and prednisolone. In this rodent model of RA, we found that the sPLA₂I reduced all measured markers of arthritis pathology and was more effective than conventional anti-arthritic treatments.

Materials and methods

Animals

Pathogen-free female Wistar rats weighing 225 to 275 g were used in this study and housed in cages with 12-hour light/dark cycles at 23°C, and 60% humidity. All animal experimentation conducted in this study was performed in accordance with National Health and Medical Research Council of Australia guidelines and with approval from the ethics committee of the University of Queensland.

Model of antigen-induced monoarticular arthritis

Rats were sensitised with methylated bovine serum albumin (mBSA; Sigma Aldrich, St Louis, Missouri, USA) in 0.5 ml Freund's complete adjuvant (Sigma, USA) as previously described [22,23]. Injections were administered subcutaneously in the rat's flanks, one at three weeks (Day -21) and the other at two weeks (Day -14) prior to arthritis induction (Day 0). At Day 0 animals were anaesthetised with ketamine (80 mg/kg, i.p.) and xylazine (12 mg/kg, i.p.). Arthritis was then induced in the right knee of each animal by aseptically injecting 0.5 mg of mBSA dissolved in 50 µL of saline into the joint space, while 50 µL of saline was injected into the left knee as a control.

The widths of both left and right knee joints were measured with a vernier caliper at regular intervals before arthritis induction and daily throughout the 14-day experiment. Following the induction of arthritis, gait impairment was assessed semi-quantitatively in each animal, by an investigator blinded to treatment groups. Animals were scored using a five-point scale (0 to 4) according to set criteria as previously described [23].

At the completion of the study, the animals were anaesthetised with intraperitoneal zolazepam (50 mg/kg) and xylazine (12 mg/kg). Blood was obtained from the inferior vena cava for the collection of serum and both left and right knee joints from animals were then dissected and fixed in 10% buffered formalin. Tissues were decalcified in a saturated EDTA solution for 21 days and embedded in wax. Sections (5 µm) were stained with hematoxylin and eosin and examined by a trained observer who was blinded to the treatments. Each tissue was scored on the degree of joint damage as previously

described [23]. Scoring was based on a scale from 0 to 8. Scores of 0 were assigned to normal joints with no detectable abnormalities while scores increasing from 1 to 8 were reserved for the graded appearance of synovial cell proliferation, fibrosis, inflammatory cell infiltration, haemorrhage and cartilage destruction [23]. These parameters were chosen as this model of antigen-induced monoarticular arthritis produces a local inflammatory response in the affected knee and minimal bone and cartilage erosion, as has been previously described [22,23].

TNF α measurement

Serum samples were assayed for TNF α concentrations using an enzyme-linked immunosorbent assay (ELISA) kit (BD Pharmingen, Franklin Lakes, New Jersey, USA). Samples were diluted 1:10 before assay was performed according to the manufacturer's instructions.

Drug preparation

The group IIa sPLA₂I (5-(4-benzyloxyphenyl)-4S-(7-phenylheptanoylamino)-pentanoic acid), was synthesised as previously described [20,21]. Leflunomide and prednisolone were sourced from Sigma (USA). These compounds were dissolved in olive oil to a final volume of 200 μ L and administered by oral gavage. The TNF α inhibitor, infliximab (Remicade; Schering-Plough, Kenilworth, New Jersey, USA) was dissolved in saline and administered to rats via a single i.v. injection [24,25].

Treatment groups

Two separate experimental trials were conducted to examine the effects of the therapeutics at preventing and also reversing disease. In the first "prevention" trial, rats ($n = 10$ per group) were orally dosed with the sPLA₂I at either 1 or 5 mg/kg beginning two days prior to the induction of arthritis (Day -2), and then daily throughout. An arthritic control group received oral vehicle (olive oil) doses only.

In the second "reversal" trial, rats ($n = 7$ to 12 per group) were treated with compounds two days following the induction of arthritis (Day +2), once significant signs of arthritis were already apparent. The treatment groups were as follows: A. sPLA₂I (5 mg/kg; $n = 7$); B. sPLA₂I (10 mg/kg; $n = 7$); C. leflunomide (10 mg/kg; $n = 10$); D. infliximab (3 mg/kg; $n = 8$); and E. prednisolone (1 mg/kg; $n = 10$). Dosages were determined from available literature (prednisolone: [24,26,27], leflunomide: [28], infliximab: [24]). All treatment groups were orally dosed daily throughout the experimental period, except for infliximab, which was administered once, as an i.v. injection on Day +2. The arthritic control group ($n = 12$) was dosed with the vehicle only. Another group of rats

(sham-operated rats; $n = 8$) did not have arthritis induced, and were dosed with the vehicle only to determine increases in knee size and weight gain due solely to growth.

Data and statistical analysis

Where indicated, values are expressed as mean \pm the standard error of the mean (SEM). Histopathological scores are presented as individual scores, with median. Data were analysed using GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, California, USA). Statistical comparisons were made using a one-way ANOVA with a Dunnett post-test, or a Mann-Whitney U test for histopathology scores. Results were considered significant when $P < 0.05$.

Results

Trial 1 (Prevention): The effect of sPLA₂I pre-treatment on joint swelling and gait impairment

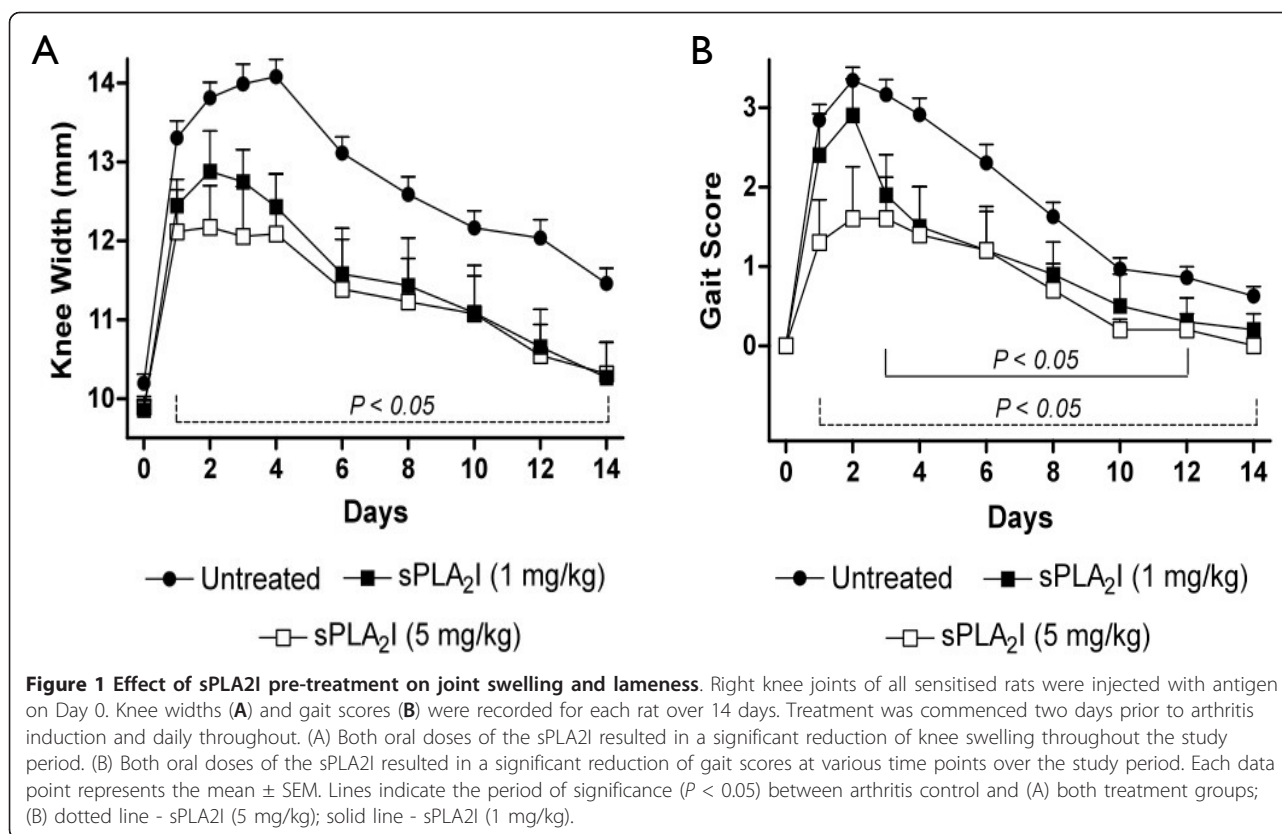
Saline-injected left knees of rats did not significantly change in width from pre-injection values during the course of each experiment (data not shown). Injection of mBSA at Day 0 to the right knees of rats resulted in a significant increase in knee widths over the 14-day study period (Figure 1A). Rats treated orally with either dose of sPLA₂I (1 or 5 mg/kg) two days prior to the injection of mBSA (Day -2), and daily throughout, had significantly reduced knee widths throughout the entire study (Days 1 to 14) compared to untreated, arthritis control rats ($P < 0.05$; Figure 1A).

The induction of arthritis in rats also resulted in considerable gait impairment which was measured via a gait score (Figure 1B). Rats pre-treated orally with the lower dose of sPLA₂I (1 mg/kg) had significantly reduced knee widths from Days 3 to 12 compared to untreated, arthritis control rats ($P < 0.05$; Figure 1B). Rats pre-treated orally with the higher dose of sPLA₂I (5 mg/kg) had significantly reduced knee widths throughout the entire study period (Days 1 to 14) compared to untreated, arthritis control rats ($P < 0.05$; Figure 1B).

Untreated, arthritis control rats also lost weight over the course of the study (Day 14 body weight: 3.0 ± 1.6 grams lost from Day 0 body weight). In contrast, rats pre-treated with the sPLA₂I (1 or 5 mg/kg) gained significant weight at the completion of the study (Day 14 body weight: 8.0 ± 2.2 and 6.5 ± 3.1 grams gained respectively from Day 0 body weight; $P < 0.05$ compared to untreated, arthritis control rats; data not shown)

Trial 1 (Prevention): Effect of sPLA₂I pre-treatment on joint histopathology

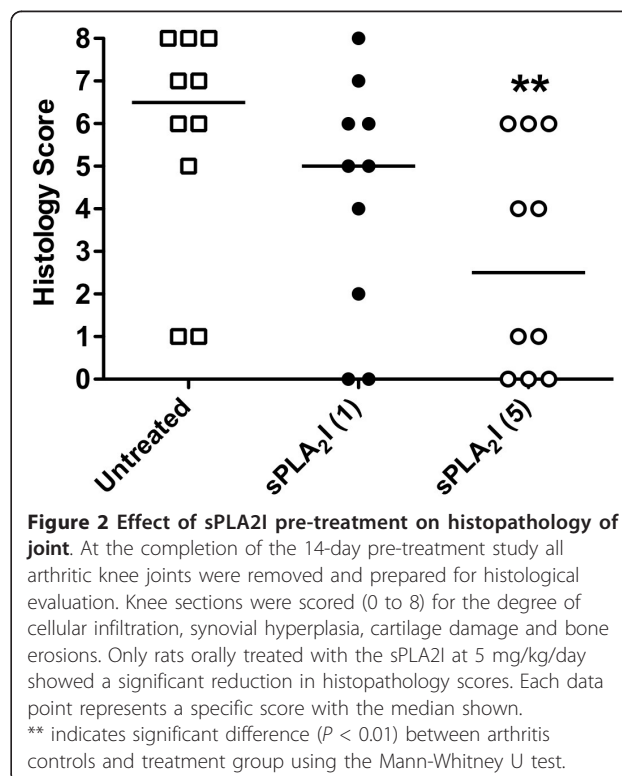
At the completion of the study, knee joints were examined histologically and scored on the degree of damage by an



experienced observer, blinded to the treatment groups (Figure 2). All saline-injected, left knees of rats had no observable pathology (data not shown). The induction of arthritis to the right knees of rats resulted in distinct cellular infiltration, which was predominantly neutrophils, with synovial cell proliferation and hyperplasia. Cartilage erosions, however, were minimal. Rats orally pre-treated with the sPLA₂I at the higher dose (5 mg/kg) had significantly reduced joint histopathology scores compared to untreated, arthritis control rats ($P < 0.05$; Figure 2). In contrast, rats pre-treated with the lower dose of the sPLA₂I (1 mg/kg) did not show a significant reduction in joint histopathology scores ($P > 0.05$; Figure 2).

Trial 1 (Prevention): Effect of arthritis induction on circulating cytokine levels

At Day 14, serum from diseased- and sham-treated rats showed no significant difference in TNF α concentrations (0.41 ± 0.02 and 0.37 ± 0.01 respectively; $P = 0.69$). These data are in accordance with previous research that describes this model as a local, rather than systemic, model of RA [22]. Therefore, it was decided not to apply this measurement to future experiments.



Trial 2 (Reversal): Effect of drug post-treatment on joint swelling and gait impairment

Treating animals two days following the induction of arthritis (Day +2) and daily throughout the study with the sPLA₂I (5 and 10 mg/kg) significantly ameliorated joint swelling throughout the entire study period following treatment ($P < 0.05$, Days 3 to 14; Figure 3A). Animals treated from Day +2 with the various comparator drugs: leflunomide (10 mg/kg), infliximab (3 mg/kg), and prednisolone (1 mg/kg) also had significant reductions in joint swelling, although for a reduced time period than the sPLA₂I ($P < 0.05$, leflunomide (Days 4 to 8), infliximab (Days 3 to 8), prednisolone (Day 10); Figure 3B).

Gait scores significantly improved following treatment with either dose of the sPLA₂I (5 or 10 mg/kg) from Day +2 ($P < 0.05$, Days 4, 10, 12; Figure 3C). Animals treated with the various comparator drugs from Day +2 also had significant reductions in gait scores, although

again for a shorter time period than the sPLA₂I ($P < 0.05$, leflunomide (Days 6, 8), infliximab (Days 6 to 10), prednisolone (Day 4); Figure 3D).

Joint swelling and gait scores were assessed over the entire trial period by comparing area under the curves from Figure 3. Overall, throughout the trial period, only sPLA₂I (5 or 10 mg/kg) showed a significant difference in both knee width and gait score (5 mg/kg: $P < 0.001$ and 0.01; 10 mg/kg $P < 0.01$ and 0.05; Figure 4). Infliximab demonstrated an overall significant decrease in knee width ($P < 0.05$) and prednisolone demonstrated an overall decrease in gait score ($P < 0.05$); leflunomide did not reach significance for either parameter ($P > 0.05$; Figure 4).

Trial 2 (Reversal): Effect of drug post-treatment on body weight loss

Rats were weighed throughout the experiment, with final day (Day +14) weights expressed as a change in

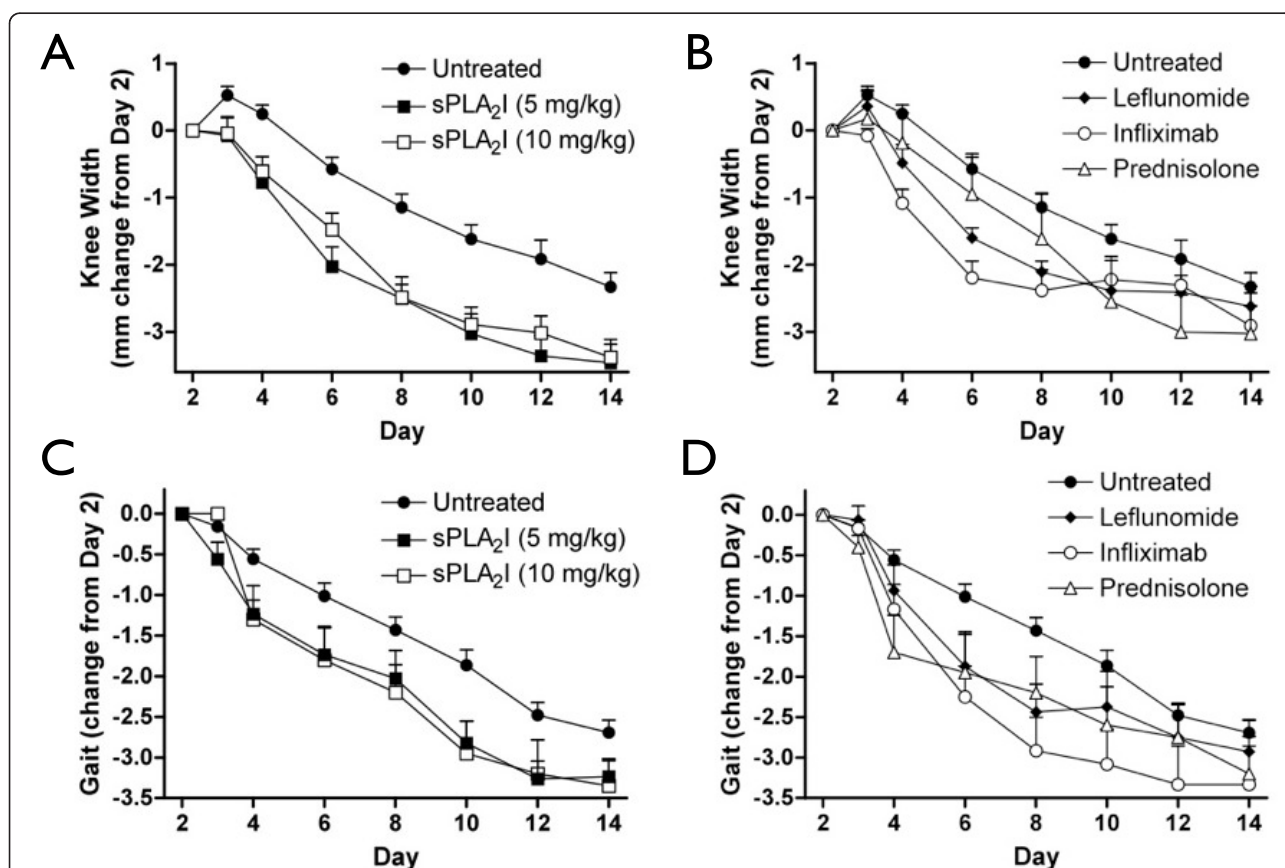


Figure 3 Effect of post-treatment on joint swelling and lameness. Right knee joints of all sensitised rats were injected with antigen on Day 0. Treatment was commenced two days following arthritis induction. Knee widths (A, B) and gait scores (C, D) were then recorded for each rat and are displayed as a change in these parameters from point of treatment (Day 2). Treatment with the sPLA₂I at both doses (5 and 10 mg/kg) resulted in a significant reduction in joint swelling (A) ($P < 0.05$; Days 3 to 14) and gait scores (C) ($P < 0.05$; Days 4, 10, 12). Treatment with the comparator drugs (leflunomide, infliximab and prednisolone) also resulted in significant reductions ($P < 0.05$) in joint swelling (B) and gait scores (D) although to a lesser extent than the sPLA₂I (knee widths: leflunomide (Days 4 to 8), infliximab (Days 3 to 8), prednisolone (Day 10); gait scores: leflunomide (Days 6, 8), infliximab (Days 6 to 10), to prednisolone (Day 4)). Each data point represents the mean \pm SEM.

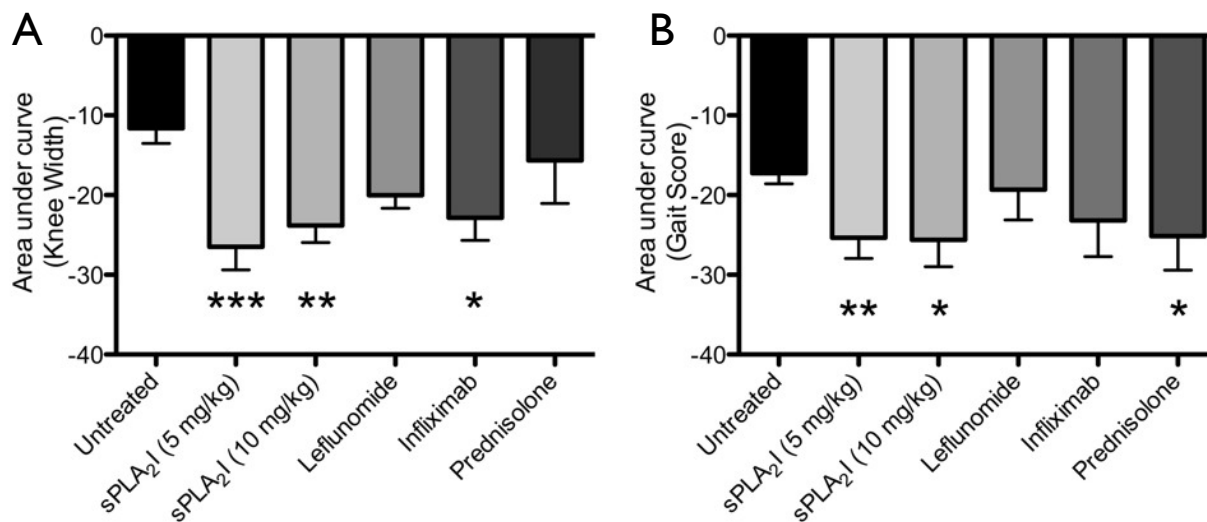


Figure 4 Effect of post-treatment on joint swelling and lameness throughout the study period. Right knee joints of all sensitised rats were injected with antigen on Day 0. Treatment was commenced two days following arthritis induction. Representative reduction in knee width (A) and gait score (B) throughout the trial period (Days 0 to 14) was attained by calculating the average area under the curve of data displayed in Figure 3. sPLA₂I treatment at 5 or 10 mg/kg achieved a significant reduction in both joint swelling ($P < 0.001$ and $P < 0.01$ respectively) and lameness ($P < 0.01$ and $P < 0.05$ respectively) over the trial period (A, B). Infliximab significantly reduced joint swelling ($P < 0.05$) without effecting lameness (A), and prednisolone significantly reduced lameness ($P < 0.05$) without effecting swelling (B). Each column represents mean \pm SEM.

weight from arthritis induction (Day 0) and compared between the various groups (Figure 5). Untreated, arthritis control rats had a total weight loss over the 14 days, and had significantly reduced weight compared to undiseased, sham-operated rats ($P < 0.05$; Figure 5). All drug treatments, with the exception of prednisolone, resulted in an increase in weight after 14 days, though none were significantly increased from untreated, arthritic control rats ($P > 0.05$; Figure 5). Prednisolone-treated rats showed considerable weight loss over the study period, and after 14 days had significantly reduced weight compared to untreated, arthritic control rats ($P < 0.05$; Figure 5).

Trial 2 (Reversal): Effect of drug post-treatment on joint histopathology

Histological analysis and scoring of diseased joints for untreated, arthritic control rats showed a similar degree of pathology compared to the same group in the first trial (Figure 6). Similarly, only rats treated with the higher dose of sPLA₂I (10 mg/kg) showed a significant reduction in histopathological scores ($P < 0.05$; Figure 6), although a reduction in median histopathological scores was seen with the lower sPLA₂I (5 mg/kg). Leflunomide treatment also resulted in a significant improvement in histopathology scores ($P < 0.05$; Figure 6). Rats treated with infliximab showed a non-significant reduction median scores, ($P > 0.05$; Figure 6)

and rats treated with prednisolone showed a clear lack of benefit, with no significant reductions in these scores ($P > 0.05$; Figure 6).

Discussion

This study is the first investigation of sPLA₂ IIA inhibition in the antigen-induced arthritis model of RA. We have previously demonstrated the usefulness of this model in establishing the efficacy of other experimental compounds and conventional anti-inflammatory drugs [23]. In the present study we have compared the efficacy of sPLA₂ group IIA enzyme inhibition, using an orally-active and specific small molecule sPLA₂I, with currently used anti-arthritis drugs in reducing antigen-induced arthritic pathology. We demonstrate that inhibition of sPLA₂ IIA alleviates the clinical signs and pathological changes associated with RA, with a greater reliability than some conventional anti-rheumatoid therapies.

sPLA₂ IIA is a secretory enzyme that converts arachidonic acid (AA)-containing phospholipids to free AA, and has been shown to be highly expressed in affected joint tissues in patients with RA [12]. sPLA₂ IIA forms the apex of an autacoids cascade in the synovium of arthritic joints. The heparin binding domain of sPLA₂ localises to lipid rafts, bringing the enzyme into close proximity to downstream mediators of this cascade, such as cyclooxygenase and lipoxygenase [29]. Additionally, sPLA₂ IIA is a ligand for the M-type receptor

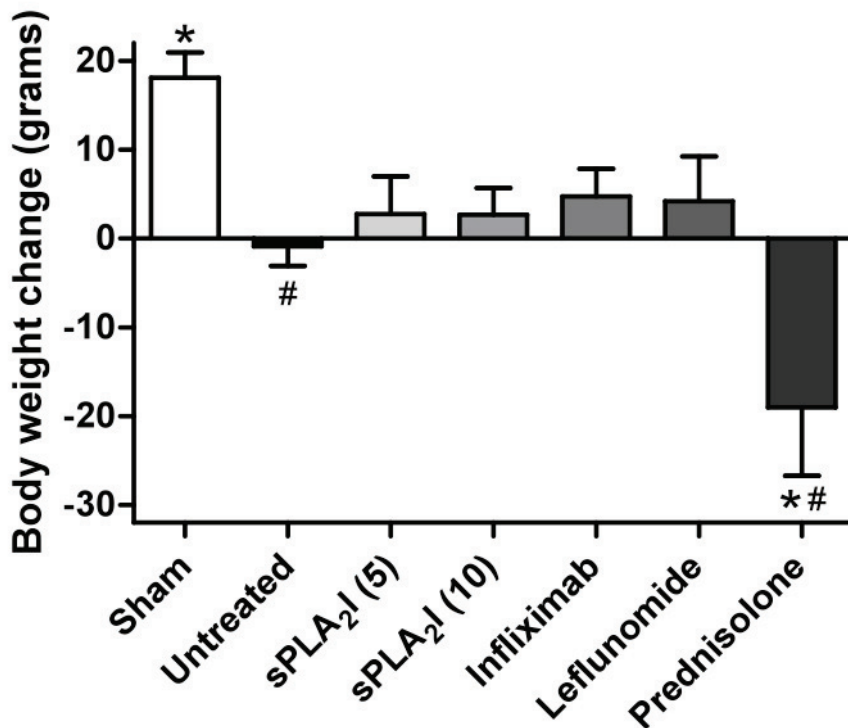
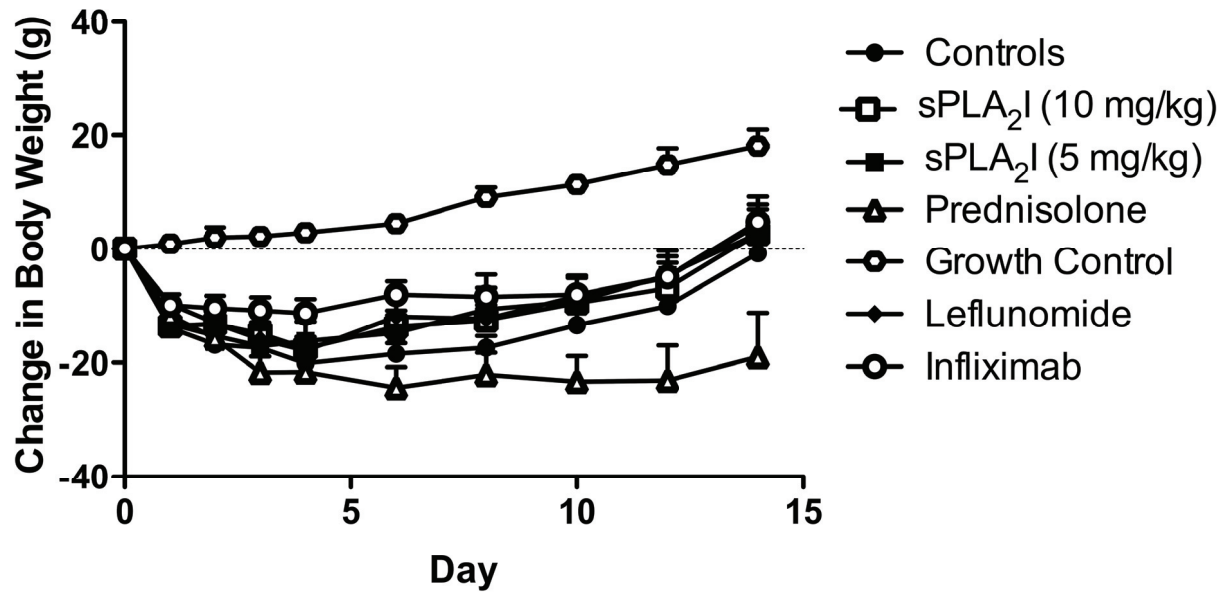


Figure 5 Effect of post-treatment on body weight. Right knee joints of all sensitised rats were injected with antigen on Day 0, and the change in body weight from Day 0 recorded every 48 hours for 14 days. Treatment was commenced two days following arthritis induction. The panels show: **(A)** Body weight over time for all treatment groups. **(B)** Change in body weight from baseline values. Arthritis control rats lost weight after 14 days, while all treatment groups gained weight, except for prednisolone-treated rats which lost considerable weight. Each data point represents the mean \pm SEM. ($P < 0.01$). Significant differences from sham-operated rats (#) and arthritis controls (*) are indicated ($P < 0.01$).

for joint swelling and gait scoring [23]. This is in contrast to leflunomide, which was able to reduce histopathology without providing an overall significant benefit to gait score and joint swelling after Day 8. Inhibition of sPLA₂ IIA, however, caused significant reductions in gait score, joint swelling and histopathology. This is evidence that each of these facets of RA is mediated by separate, but communicating, mechanisms. sPLA₂ inhibition reduces prostaglandin synthesis by COX, through reducing the concentration of free AA, whilst ibuprofen is a direct inhibitor of COX. This inhibition of prostaglandin synthesis, which both reduces inflammation and inhibits pain, may account for the significant difference seen in gait scores/joint swelling with both these drugs. Inhibition of sPLA₂ IIA also reduces mast cell degranulation and neutrophil infiltration by preventing binding to M-type receptors [33]. This attenuation of immune cell function may mimic the mast cell-specific repression of leflunomide, which causes cell cycle arrest and mast cell apoptosis, in alleviating joint histopathology [33]. The multiple actions of sPLA₂ IIA in arthritis, and its absence in the synovium

of healthy joints, are likely the reason for its success in this study when compared to conventional therapeutics. In addition, conventional therapeutics, with the exception of prednisolone, target single downstream mediators of sPLA₂ IIA actions.

Specific inhibition of sPLA₂ enzymes has an advantage over conventional RA therapeutics, in that it targets multiple possible pathways of RA pathogenesis (Figure 7), without affecting normally occurring biological processes. There have been no demonstrated side effects of sPLA₂ IIA inhibition in animal models of disease and the phase II clinical trial of an sPLA₂I in humans provided evidence of some liver toxicity only at the highest doses (1,000 mg/day) [17]. Conversely, all conventional therapeutics in this study failed to show significant benefit to every pathology measurement of the antigen-induced arthritis model. In addition, many of the conventional therapeutics have off-target effects that mitigate the benefits provided. Leflunomide and infliximab have been shown to inhibit osteogenic cell proliferation [36]; because of this, both treatments pose additional risks for post-menopausal women. Leflunomide and prednisolone

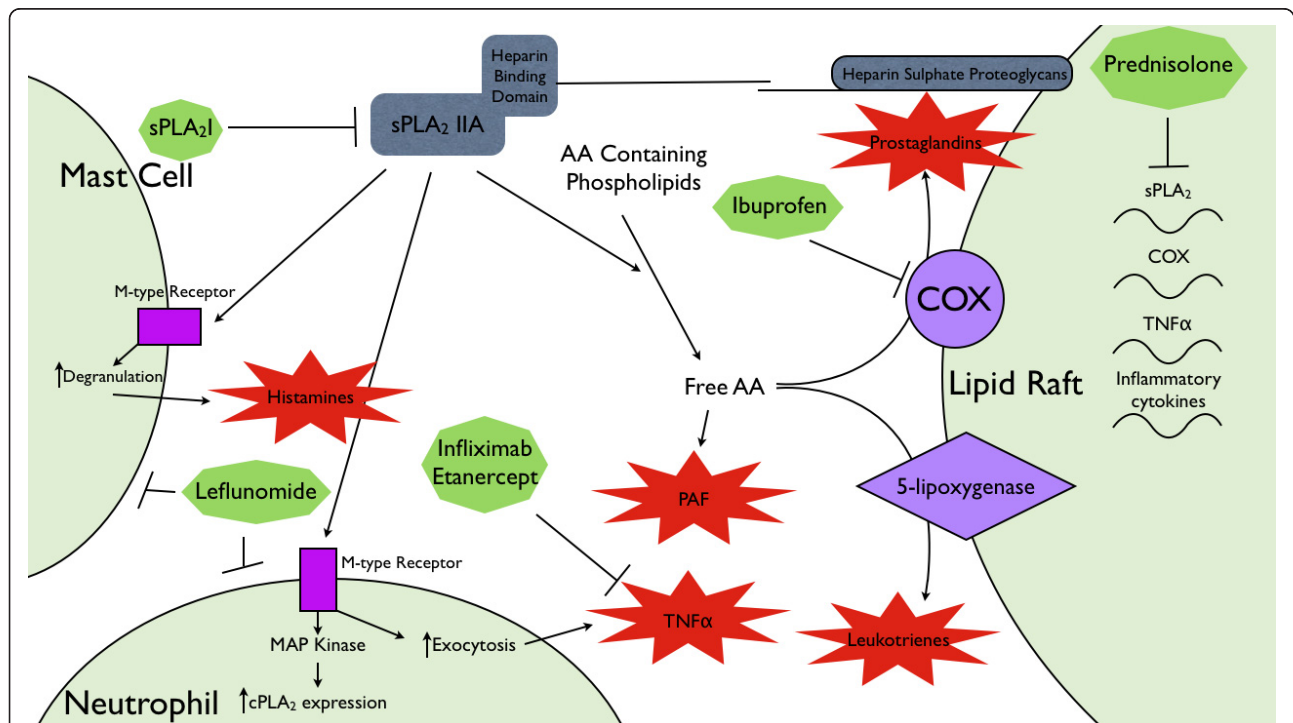


Figure 7 Simplified hypothetical model demonstrating the sites of action of anti-arthritis therapeutics used in this study. sPLA₂ IIA provides the substrate for the production of the inflammatory mediators; platelet-activating factor (PAF), prostaglandins and leukotrienes. Additionally, sPLA₂ IIA is a ligand for the M-type receptor: sPLA₂ IIA agonist activity causes mast cell degranulation and activation of neutrophils. Ibuprofen, leflunomide and infliximab all act on inflammatory mediators downstream of sPLA₂ IIA actions. Prednisolone globally alters inflammatory gene transcription. AA, arachidonic acid; cPLA₂, cytosolic phospholipase A₂; PAF, platelet activating factor; MAP kinase, mitogen activated protein kinase; M-type receptor, muscle type receptor; sPLA₂ IIA, secretory phospholipase A₂ group IIA; TNFα, tumour necrosis factor alpha.

actively suppress the immune system; leflunomide induces the apoptosis of several types of immune cells [33,37,38], and prednisolone induces cell cycle arrest, thereby inhibiting cellular proliferation. This study also confirms a catabolic effect of daily prednisolone administration as has been previously shown [24] and, although prednisolone can act as an inhibitor of sPLA₂ IIA transcription [39], the use of a low-dose steroid in a chronic condition can potentiate morbidity for the patient [40-42].

Previously, the efficacy of sPLA₂ IIA inhibition has been tested in phase II clinical trials as an adjunct to DMARD therapy without success [17]. The administration of DMARDS in conjunction with the sPLA₂I may have masked any benefits provided by sPLA₂I. Here we show, for the first time, that sPLA₂ IIA inhibition has the potential to be a useful monotherapy for the treatment of RA, and may be a more effective chronic therapy than the conventional RA therapeutics.

Conclusions

We have previously shown the sPLA₂I used in this study to be an orally-active, highly selective drug for the treatment of intestinal ischemia reperfusion injuries [19] and inflammatory bowel disease [20] in rat models, and now demonstrate its efficacy in a model of RA. Inhibition of sPLA₂ IIA using a chemically different sPLA₂ enzyme inhibitor, has previously been trialed in human RA. In this clinical trial, the sPLA₂ inhibitor was administered as an adjunct therapy to DMARD and glucocorticoid therapy, which may have masked any benefits to patients [17]. By directly comparing sPLA₂ inhibition to conventional therapies in a rodent model of antigen-induced arthritis, we have provided a rationale and evidence for the use of sPLA₂I as a replacement for DMARD/glucocorticoid therapy in future clinical trials.

Abbreviations

AA: arachidonic acid; DMARD: disease modifying anti-rheumatic drug; mBSA: methylated standard error of the mean; PAF: platelet activating factor; sPLA₂: secretory phospholipase A₂; sPLA₂I: secretory phospholipase A₂ inhibitor; TNFα: tumour necrosis factor alpha.

Acknowledgements

These studies were supported in part by a grant from the National Health and Medical Research Council of Australia. We thank Prof David Fairlie for supplying the sPLA₂I, and Dr Nathan Dryburgh for assisting in some of the experiments in this study. We also thank Mr Paul Addison for expert technical assistance in histological preparation of knee samples.

Authors' contributions

TW, IS and ST participated in the design of the study and interpretation of data. TW, JC, BR and LC participated in the acquisition and analysis of the data. IS performed the histopathological analysis and scoring of the knee joints. TW and LC drafted the manuscript and ST and IS assisted in editing the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 10 November 2010 Revised: 10 February 2011

Accepted: 14 March 2011 Published: 14 March 2011

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doi:10.1186/ar3278

Cite this article as: Coulthard et al.: Comparative efficacy of a secretory phospholipase A₂ inhibitor with conventional anti-inflammatory agents in a rat model of antigen-induced arthritis. *Arthritis Research & Therapy* 2011 **13**:R42.

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