# Inhibitors of Tumor Necrosis Factor Synthesis as a New Approach for the Treatment of Rheumatoid Arthritis

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## Abstract

**Objective:** The treatment of rheumatoid arthritis (RA) is based on the inhibition of TNF. Here we evaluated whether drugs that might inhibit TNF, such as pentoxifylline (PTX), rupatadine (RUP), rolipram (ROL) and thalidomide (THA), could be an alternative for RA treatment.

**Methods:** In wistar male rats the changes in paw thickness, plasma TNF and by the activity of basic aminopeptidase (APB) in soluble fraction of synovial tissue and peripheral blood mononuclear cells (PBMC) evaluated after daily injection for 30 days were taken as anti-inflammatory outputs, while hepatotoxicity was assessed by measuring plasma alanine transaminase (ALT) and aspartate transaminase (AST) activity. The content of IL1- , IL-6 in serum and synovial fluid and the histology of the injured tissue were determined only for ROL, THA and ROL+THA. Prednisolone was used as a standard drug.

**Results:** Collagen treatment induced paw thickness, histological changes in the tibiotarsal joint, increase in synovial fluid of both cytokines and synovial tissue of APB activity. Furthermore, the APB activity in PBMC was reduced and ALT and AST activity were enhanced. The most effective drug schedule in reducing arthritis induced changes described above, as well as recovering from control levels TNF, IL1-β, APB in synovial tissue and AST activities were THA and the association of ROL and THA. However, only THA alone reduced the levels of ALT.

**Conclusion:** The synthesis of TNF in RA models can be blocked by drugs acting at different targets. We show that THA and THA+ROL emerges as simple and effective therapeutic alternatives for RA.

**Keywords:** Rheumatoid Arthritis • TNF • Anti-TNF • Basic Aminopeptidase • Thalidomide • Rolipram • Drug Repositioning

## Introduction

Rheumatoid arthritis (RA) is a systemic, chronic, and autoimmiune inflammatory disease of unknown etiology, which affects approximately 1% of the world population. Collagen induced arthritis (CIA) is a classical model for studying RA [1,2]. Pro-inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin (IL)-1β and IL-6 are found in high concentration in the blood and synovial fluid of human with RA and CIA rats [2-4]. TNF, a pivotal player in chronical inflammatory diseases is involved in the processes of differentiation, maturation, and recruitment of osteoclasts, leading to destruction of bone and articular cartilage [1,2,5]. Additionally, in CIA rats, basic aminopeptidase (APB) activity decrease in the soluble fraction of peripheral blood mononuclear cells (PBMCs) and increase in the same fraction of synovial tissue were considered as biomarker for CIA [6,7].

The current treatment of RA is based on corticosteroid therapy, being prednisolone the standard drug (8) and TNF antibodies a therapy available for severe cases [1]. However, the therapy with corticosteroids is not selective, and the TNF antibody therapy that focus on reducing the availability of the key pro-inflammatory signal in RA, have important adverse effects, lack manufacturing reproducibility and high cost [5,8-10] . In this study we pursue the same goal by using drugs that interfere in different stages of TNF synthesis: pentoxifylline (PTX), rolipram (ROL), rupatadine (RUP) and thalidomide (THA) [11-14].

These drugs, which have been evaluated clinically, are classically used in the treatment of other diseases and, except for RUP, have already been partially evaluated for the therapy of RA [14-24]. For the best of our knowledge, only 6 clinical studies that evaluated the possible antiarthritic actions of THA. The data are controversial, while some studies showed remission [19,20], others presented beneficial effects but with side effects [23,24]. The other 6 clinical studies evaluated the effects of PTX in RA, showing improvement of disease in its use alone [18,22] or combined with methotrexate [16] or etanercept [21]. No clinical studies tested the antiarthritic effects of RUP and ROL.

In this study were evaluated the effect of these drugs, that potentially inhibits TNF, in reducing the development and severity of the disease in CIA rat model.

**Hypothesis:** Knowing the involvement of TNF in RA and which anti-TNF biological drugs are being adopted after ineffectiveness of synthetic drug therapy, which is the first indication for RA patients, and which does not primarily target TNF, this paper aims to investigate the antiarthritic potential of synthetic drugs that inhibit TNF to assist in alternative therapeutic treatments.

## Material and Methods

### Animals, treatments and samples

Wistar male rats (160-180g), from Animal Facility of Butantan Institute, São Paulo, Brazil, were kept at 25°C, relative humidity of 65.3 ± 0.9%, with ad libitum water and food access. The animals were housed in 12h light/12h dark cycle (lights on at 06:00 AM), and the experimental procedures were performed between 9:00-11:00 AM. The protocols were approved by the Committee on Ethics in the Use of Animals of Butantan Institute (protocol n°1040/13). The number of experimental and control groups varied according to obtained arthritic animals, already that only 60-70% of induced animals developed the disease. All experiments were executed with C-S and AR animals in the same period.

### Induction of arthritis and treatments with drugs

The process of inducing arthritis was based on the method of Chen et al [25]. Animals anesthetized with ketamine/xylazine (75 mg/kg of ketamine and 1 mg/kg of xylazine, intraperitonially-i.p.) were injected intradermally (i.d.) with type II collagen (CII) (2 mg/mL) emulsified in incomplete Freund's adjuvant (FA) (day zero). At day 7, 100 µL/animal of the same emulsion (CII+FA) was administered via i.d. in four different regions; the tail, and in the 3 positions in the back, control animals received saline (0.9% NaCl) at the same positions. The treatments began on day 14.

The animals were then classified according to the score scale proposed by Erlandson-Harris [26] based on the plantar thickness of the hind paws, presence of erythema and cyanosis. 70% of the animals reached the criteria, and were admitted in the study. The animals that reached the criteria were injected from the 14$^{th}$ till the 44$^{th}$ day with PRED (prednisolone from Biosynthetic); PTX (Pentoxifylline from EMS); ROL (subcutaneous, 3 mg/Kg, Rolipram from Cayman Chemical Company); THA (Thalidomide from FUNED), or RUP (Rupafin from Pharmaceutical Biosynthetic). The controls included animals that did not receive CII+FA; and those that developed rheumatoid arthritis and were treated with vehicle of the drugs; distilled water for RUP and PTX and 2% DMSO for THA and ROL. Animals were killed on day 45.

ROL and THA were administered on bolus on the back, subcutaneously (s.c), at doses of 3 mg/kg [14,17] and 30 mg/kg [13,27] respectively, at a maximum volume of 200 µL/animal. PRED, PTX and RUP were administered by oral gavage at a dose of 5 mg/kg [28], 100 mg/kg [27,29] and 0.0285 mg/kg (30) respectively, at a maximum volume of 600 µL/animal. The 0.9% saline was administered on the back via s.c. and tap water by gavage, respectively at maximum volumes of 200 and 600 µl/animal, in AR and controls (C-S). All treatments were performed for 30 days and on the 45th day after the first injection the animals were anesthetized as previously described for collection of the material specified below. Rats not inserted in any of these experimental groups were euthanized, under anaesthesia by decapitation.

The doses of the drug were selected according to the current use for experimental treatment of arthritis and/or other inflammatory diseases in rats [27].

### Sample collection

Blood was collected by cardiac puncture in syringes heparinized or not for obtaining plasma and the soluble fraction of PBMC's, or the serum, respectively [7]. Synovial tissue (ST) and its soluble fraction were obtained from the tibiotarsal joint of the left hind paw, as previously described [6,7,30-32], and right tibiotarsal joint was histologically characterized [32].

## Assessment of arthritis and hepatotoxicity

### Macroscopic evaluation

It was performed by observing the erythema (redness), cyanosis (purplish) and quantification of the thickness of the plantar region with pachymeter (Mitutoyo, USA) by means of the mean plantar thickness measurement in the medial region of the hind legs. The score proposed by Erlandsson-Harris et al. [33] was applied in order to evaluate arthritis degree in rats.

### Histological analysis of the tibiotarsal joint

Samples were fixed in 10% formaldehyde for 7 days and transferred to 70% ethanol for additional 7 days. Decalcification was performed in 10% nitric acid solution for 72 hours, fixed in 10% formaldehyde for 48 h and dehydrated for 1h in 70% alcohol, 1 h in 96% alcohol and 1h in absolute alcohol. After dehydration, the samples were left for 2 hours in xylene and then placed in paraffin bath for 3 hours. The sections were cut lengthwise in a thickness of 5 micrometers for the assembly of the slides stained with hematoxylin-eosin for analysis under an optical microscope. The analysis was performed with a Nikon E600 microscope equipped with CoolSNAP-PRO® digital camera, using Image-Pro Plus® 4.0 software (Cybernetics).

### Alanine transaminase, Aspartate transaminase, TNF, IL-1β e IL-6 measurement

ALT and AST were measured in plasma using commercial colorimetric kits (Laborclin, Brazil). TNF (ELISA Kit for rat, Merck, Germany) was measured in plasma, while IL-1β and IL-6 (ELISA Kit Raybiotech, USA) were measured in the serum and synovial fluid.

### APB activity and protein content

Both methods were used for the soluble fraction of synovial tissue and PBMC. Protein content was measured by Bradford method with a Bio-Rad protein assay kit (Bio-Rad) using BSA dissolved in the same sample diluent as standard. APB activity was quantified based on the amount of β-naphthylamine released as the result of incubation of samples with β-naphthylamide (Sigma), being expressed as picomoles of hydrolyzed substrate per minute per milligram of protein [6,31]. Both were read in Bio-Tek FL600FA microplate fluorescence/absorbance reader (BioTek, Winooski, VT, USA)

## Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM), and differences between two samples were tested by Student's t-test, while differences of more than two samples were tested by analysis of variance (ANOVA), followed by the Student-Newman-Keuls or Bonferroni post-hoc tests. The level of significance was $p<0.05$, and the data were calculated with the GraphPad Instat™ software.

## Results

Here we evaluated the effect of non-classical anti-inflammatory drugs in a rat model of AR induced by sequential administration of CII and FA. The body mass of the control and AR animals did not vary along the 45

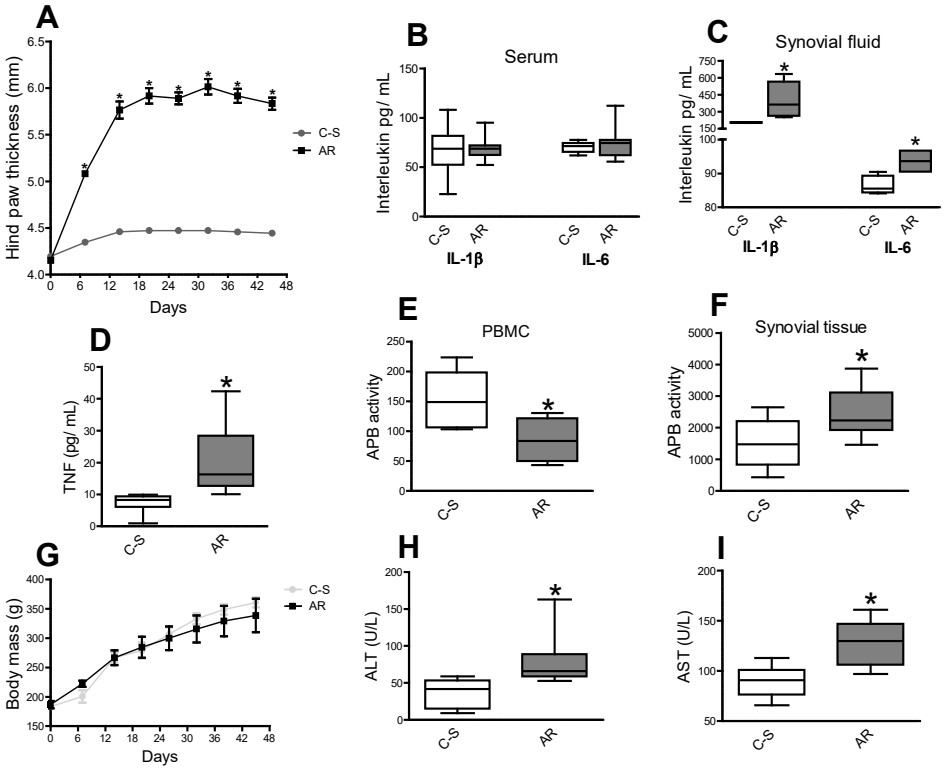


**Figure 1.** Characterization of the development of CIA arthritis in AR compared to C-S. A) Time course of thickness of the plantar region of the hind paws; B) Interleukin (IL)-1β and IL-6 in serum; C) IL-1β and IL-6 in synovial fluid (LS); D) TNF in plasma; E) APB in soluble fraction (SF) of synovial tissue (ST) (pmoles of hydrolyzed substrate, min-1 mg protein-1); F) Activity of APB in SF of peripheral blood mononuclear cells (PBMCs); G) Time course of body mass (g); H) Plasmatic levels of alanine-transaminase (ALT); I) Plasmatic levels of aspartate-transaminase (AST); A-I) Values are mean±SEM. Number of animals A/G:C-S/AR= 9; B:C-S=12/AR=11 (IL-1β);C-S/AR=7 (IL-6); C:C-S=4/AR=5 (IL-1β);C-S/AR=4 (IL-6); D: C-S=9/AR=8; E:C-SAR=8; F:C-S/AR=12; H:C-S/AR=16; I:C-S/AR=13. *p<0.05vsC-S.

days (Figure 1A). The increase in the mean plantar thickness of the hind paws of AR animals followed a hyperbolic curve. At day 6 the values were already significantly different from day 0 and from rats injected for 6 days with saline. The maximum increase obtained at day 24 was maintained till day 45 (Figure 1B). Plasma TNF values, as well as, ALT and AST activity increased in AR when compared to controls, while serum IL-1 and IL-6 did not vary (Figure 1C-Figure 1E). Otherwise, in the soluble fraction of the synovial tissue, IL-1 and IL-6 was significantly increased at day 45 (Figure 1F).

In the present model prednisolone (5 mg/Kg) reduced plantar thickness, (Figure 2A), APB activity in the soluble fraction of PBMC and

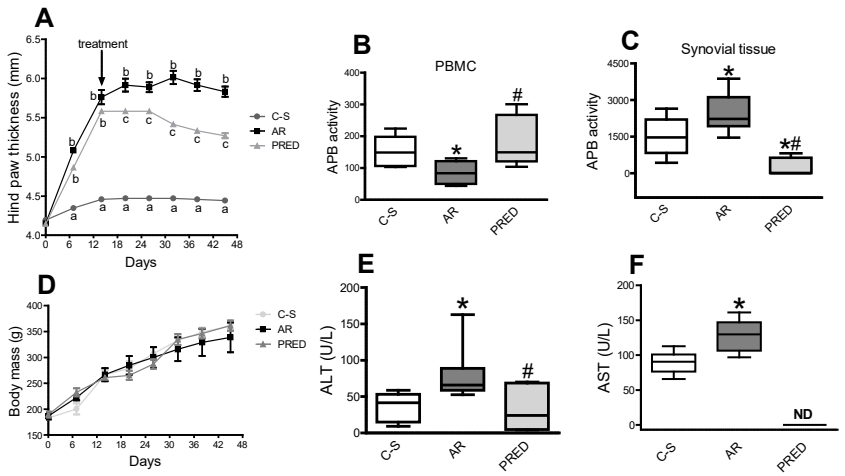


**Figure 2.** Characterization of the antiarthritic action of PRED treatment, compared with C-S and AR. A) Time course of thickness of the plantar region of the hind paws; B) Activity of the basic aminopeptidase (APB) in soluble fraction (SF) of synovial tissue (ST) (pmoles of hydrolyzed substrate, min-1 mg protein-1); C) Activity of APB in SF of peripheral blood mononuclear cells (PBMCs); D) Time course of body mass (g) before and after CIA induction; E) Plasmatic levels of alanine-transaminase (ALT); F) Plasmatic levels of aspartate-transaminase (AST); A-I) Values are mean±SEM. Number of animals A/B/D-F:PRED=6; A/D: C-S/AR=9; B:C-S/AR=8; C:C-S/AR=12;PRED=5; E:C-S/AR=16; F:C-S/AR=13. Analysis of variance (ANOVA) and Student-Newman-Keuls (C-SxARxPRED), *p<0.05vsC-S and #p>0.05vsAR.

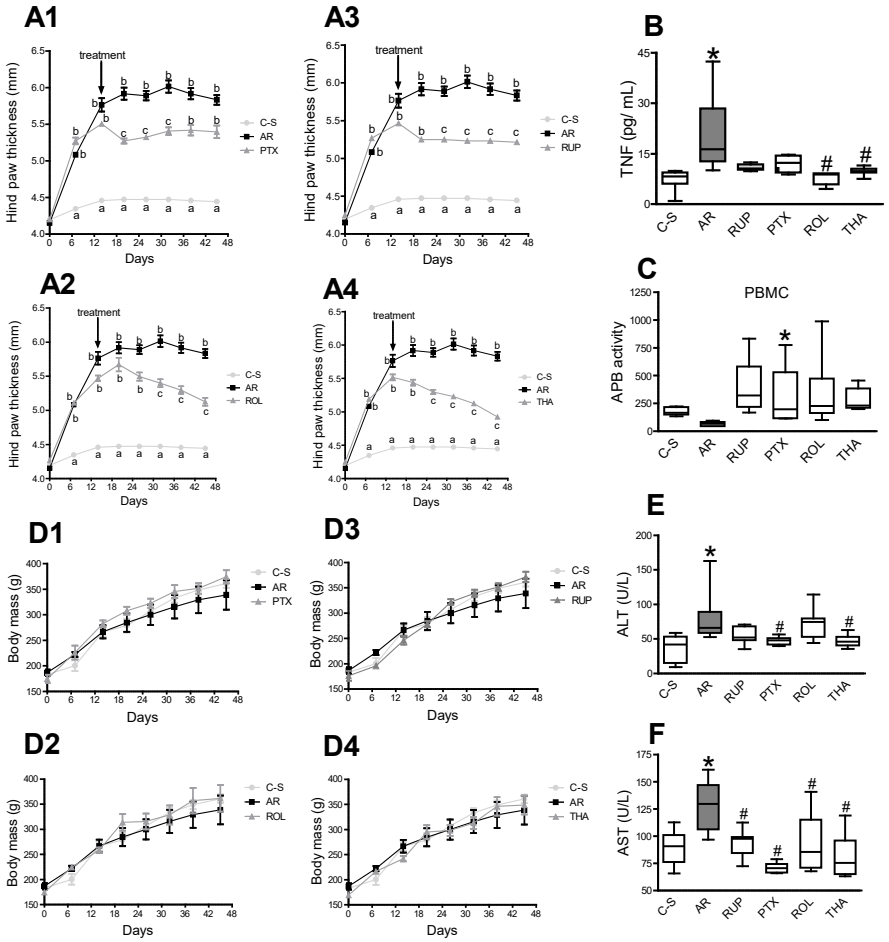


**Figure 3.** Characterization of the antiarthritic action of PTX, ROL, RUP and THA compared to C-S and AR. A) Time course of thickness of the plantar region of the hind paws; B) Tumor necrosis factor (TNF) in plasma; C) Basic aminopeptidase activity (APB) in the soluble fraction (FS) of synovial tissue (TS) (pmoles hydrolyzed substrate per min-1 per mg of protein-1); D) Time course of body mass (g) C-SxARxtreatment (RUP, PTX, ROL or THA); E) Plasmatic levels of alanine-transaminase (ALT); F) Plasmatic levels of aspartate-transaminase (AST); Values are mean ± SEM. Number of animals in items A/D:C-S/AR=9, PTX=6, ROL/THA=8, RUP=7; B:C-S=9;AR=8;PTX=4;ROL=7;RUP=5;THA=8; C:C-S/AR=12;PTX/ROL/RUP/THA=6; E:C-S/AR=16; F:C-S/AR=13; E/F:PTX=6;ROL/THA=8; RUP=7. Analysis of variance (ANOVA) and Bonferroni for multiple paired comparisons (C-S/AR vs treatment groups), *p<0.05vsC-S and #p>0.05vsAR.

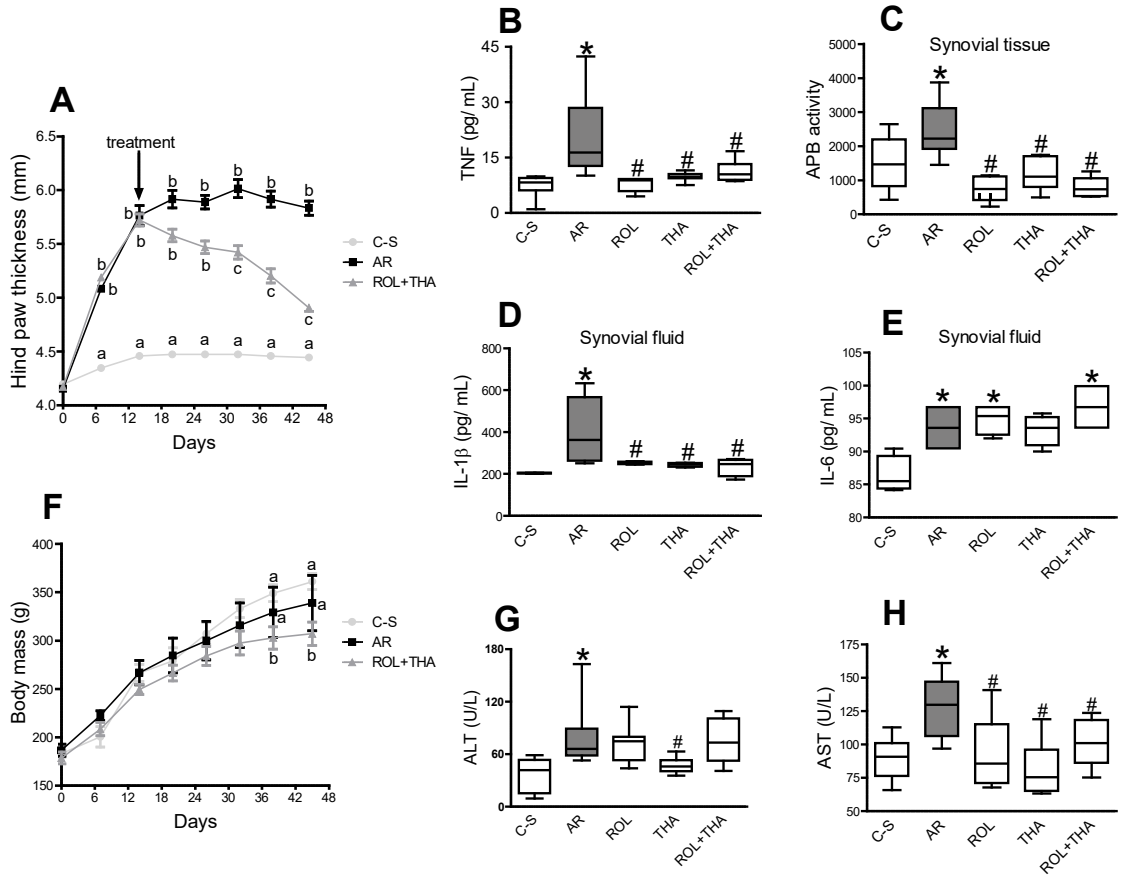


**Figure 4.** Characterization of the antiarthritic action of the treatments with ROL, THA ROL+THA compared to C-S and AR. A) Time course of thickness of the plantar region of the hind paws; B) TNF in plasma; C) APB in soluble fraction (FS) of synovial tissue (ST); ANOVA, p= 0.0356; D) Interleukin (IL)-1β in synovial fluid (LS); E) IL-6 in synovial fluid (LS); F) Time course of body mass (g); G) plasma alanine-transaminase (ALT); ANOVA, p<0.0001; H) Plasma aspartate-transaminase (AST); ANOVA, p<0.0001. A-E) Values are mean±SEM. Number of animals in A/F:C-S/AR=9;ROL+THA=8; B:C-S=9;AR=8;ROL=7; THA=8;ROL+THA=6; C:C-S/AR=12;ROL/THA/ROL+THA=6; D:C-S/ROL/THA=4;AR/ ROL+THA=6; E: all groups=4; G:C-S/AR=16; H:C-S/AR=13; G/H:ROL/THA=8; ROL+THA=7. Analysis of variance (ANOVA) and Bonferroni for multiple paired comparisons (C-S and AR vs treatment groups), *p<0.05vsC-S and #p>0.05vsAR.

synovial tissue (Figure 2B-Figure 2D) and plasma AST activity (Figure 2E). Additionally, the treatment reduced ALT (Figure 2F) levels to values far below the control group. Thus, the model reacts to a classical anti-inflammatory drug accordingly to the literature [32].

The treatment with RUP, ROL and THA caused a reduction on swelling of hind paws (Figure 3A) after 12 (RUP e THA) and 18 (ROL) days, while PTX reduced the edema in 6 days (Figure 3A1-Figure 3A4). After 24 days, PTX reduced paw thickness when compared to AR animals treated with saline, and did not change the size of naïve animals (Figure 3A). Body mass was not affected by any treatment (Figure 3D1-D4). RUP and PTX have no effect on the increase in TNF in AR, but reduced the values of AST, while only PTX affected the increase in ALT. On the other hand, ROL and THA reduced plasma level of TNF and AST to those observed in naïve animals treated with saline, while only THA restored ALT levels.

## Evaluation of Selected Drugs

Although all the drugs reduced hind paw swelling when compared to AR (Figure 4A-Figure 4H), only ROL and THA reduced TNF, therefore these drugs were selected for a more detailed evaluation by analyzing the concomitant administration of both drugs. This new protocol did not improve any of the parameters presented above (hind paw size, plasma TNF level and AST activity, APB, ALT activity in soluble fraction of synovial tissue, but reduced the body mass.

The determination of the level of IL-6 and IL-1b in the synovial fluid showed a significant reduction of these pro-inflammatory cytokines by the isolated and the combined ROL and THA. Interestingly, both ROL and THA alone did not lead to changes in body mass, while, ROL+THA reduced body mass.

## Histology

As can be observed in the representative images, in AR animals (Figure 5I-Figure 5V), the most evident histopathological changes observed, compared to C-S (Figure 5I), were bone and cartilage erosion, cellular infiltration in the joint cavity and synovial hyperplasia after 45 days of induction. ROL treatment improved cellular infiltration in the joint cavity and partially reverted erosion of bone and cartilage (Figure 5-III). Treatment with THA (Figure 5-IV) improved bone and cartilage erosion and fully recovered cellular infiltration in the joint (Figure 5-II). The combined treatment of ROL+THA (Figure 5-V) greatly improved bone and cartilage erosion and cellular infiltration in the joint (Figure 5-II).

## Discussion

Arthritic animals in the CIA model can be differentiated macroscopically from healthy animals by the presence of erythema, cyanosis and severe paw swelling, a condition that persisted for 30 days. Histologically, bone and cartilage erosions, as well as increased cellular infiltration and synovial hyperplasia confirmed the development of arthritis in the tibiotarsal articulation [34].

We tested the efficacy of prednisolone and drugs with predicted anti-TNF effects in the CII-adjuvant arthritis animal model. Firstly, we observed that only ROL and THA decreased the levels of circulating TNF in AR animals and, similar to PRED treatment, ROL and THA, alone or in combination, reduced the paw inflammatory lesion. The treatments with RUP and PTX did not reduced the lesion but partially stabilized the disease progression. The treatment with THA, RUP and PTX, but not with ROL, restore the levels of synovial APB, AST and ALT. Interestingly, the ROL+THA treatment also reduced the weight gain of the AR animals. Additionally, the present study shows a hepatoprotective effect of THA, but not ROL, since the treatment recovers the levels of transaminases to normal values.

The peptide activity of APB occurs on substrates with recognized actions in the regulation of the angiogenic process [35] and participates in the maturation of peptide hormones and neurotransmitters [36]. In addition, previously evidences showed that APB activity is a marker of the development of arthritis in the CIA model in rats [6,31]. The present

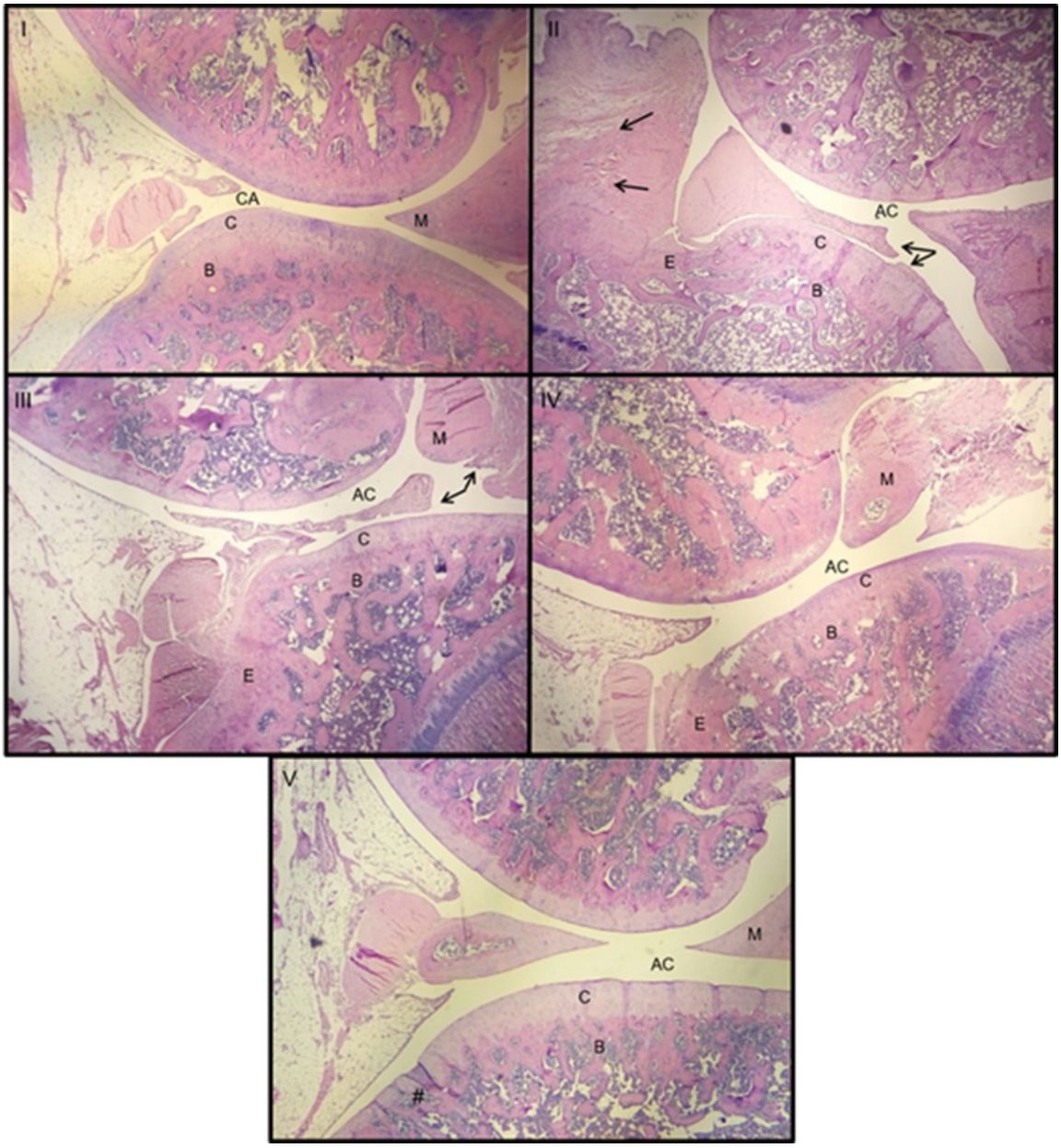


**Figure 5.** Histology of the tibiotarsal joint in longitudinal section of control (I), arthritic treated with saline (II) and treated with ROL (III), THA (IV) or ROL+THA (V) after 15 days of induction and 30 days of treatment (day 45). Cartilage (C), bone (B), intra-articular cavity (AC), meniscus (M), bone (BE) and cartilage erosions (CE) in ROL and ROL+THA treatments. Arrows indicate cellular infiltration sites in the AR group and # shows erosion improvement in ROL+THA treatment. HE.

study confirms the same profile of APB activity (decrease in the soluble fraction of PBMCs and increase in this same fraction of synovial tissue) in AR compared to C-S animals. All treatments inhibit APB activity in the soluble fraction of synovial tissue but not in the soluble fraction of PBMCs, suggesting that the treatments act at the primary inflammatory focus of the disease. Corroborating this idea, IL-1β levels were also reduced locally after the ROL, THA and ROL+ THA treatments. In addition, it is possible to hypothesize that the local inhibition of APB results in the reduction of angiogenesis, chemotaxis and neutrophil stimulation [35], being beneficial in the treatment of RA. Therefore, the monitoring of APB activity in RA patients can be an inexpensive, simple and effective parameter for the prognosis, diagnosis and monitoring of RA. Interestingly, although prednisolone has known anti-inflammatory actions in AR, the treatment with the drug reduced APB to levels lower than the control group. This effect, might be considered carefully because the basal activity of this enzyme is required for other important physiological functions [36].

The increase of alanine and aspartate transaminases in blood stream is a marker of liver damage [37]. Because AST is also present in the muscle and heart tissues, diagnosis of hepatotoxicity requires a concomitant measurement of ALT [37]. As previously observed for Freund's complete adjuvant-induced RA [38], levels of ALT and AST are also elevated in our CIA model. There are reports of a possible hepatoprotective property of THA [39], an effect similar to what was observed in the present study in relation to both transaminases. Antirheumatic drugs are among the medications commonly associated with adverse hepatic reactions or hepatotoxicity [40]. A systematic review on methotrexate (MTX)-a first-line drug in the treatment of RA [41, 42], showed that 13% out of 3808 patients presented twice the normal limit levels of ALT or AST and 3.7% of patients discontinued treatment due to hepatotoxicity [42]. Other drugs used in the treatment of RA also cause elevated levels of transaminases, among them one can mention some disease modifying drugs like sulfasalazine, diclofenac, leflunomide and azathioprine, and three biological drugs that inhibit TNF, as adalimumab, etanercept and infliximab [40]. Therefore, drugs that prevent hepatotoxicity must be considered as therapeutic alternatives. In this sense, THA emerges as a strong candidate for AR patients with liver impairment.

Another important measure in the overall assessment of drug testing is the body weight. Glucocorticoids can lead to sodium retention and loss of potassium, resulting in accumulation of body fluid and increased body mass. Prednisolone has a known adverse effect of body mass gain [43]. This effect of chronically administered prednisolone is not observed in the present study, suggesting that a good balance between dose and expected clinical outcome could be achieved. Doseyici et al. [44] showed that ROL treatment in Wistar rats with obesity induced by the hyperlipid diet caused lower gain of body mass. In addition, two *In vitro* studies have shown that ROL stimulates lipolysis [45,46]. In Swiss mice obese for hyperlipidic

diet, an administration of THA induced in adiposity accompanied by the reduction of TNF, leptin and infiltration of macrophages [47]. Another study with obese mice on a hyperlipidic diet, treated for 10 days with THA found a reduction in body mass [48]. The present study shows that none of the drugs evaluated when administered alone, affect body mass, suggesting that the decreased body mass gain observed after ROL+THA treatment is due to their combined effect.

Inflammatory cytokines such as TNF, IL-1β and IL-6 play important roles in the pathogenesis of RA[2] and have been found in high levels in serum of arthritic patients [2] and of rats with adjuvant-induced arthritis [4]. In addition to the macroscopic increase in the thickness of the hind paws and histological evaluation confirming the development of RA, the present study shows an increase in TNF plasma levels and of IL-1β and IL-6 levels in the synovial fluid, as occurs in humans with rheumatoid arthritis [2,3]. Tissi et al. [49] quantified serum and local TNF, IL-1β and IL-6 for several periods of time after induction of the diffuse septic arthritis model by intravenous injection of streptococci in mice [49] and the results suggested that levels of IL-1β and IL-6 in the joints exceeded those detected in the serum and that these cytokines remain high only during the establishment of the disease. Silva et al. [50] observed a similar profile in serum levels of TNF and IL-1β after induction of Freund's complete adjuvant arthritis, with a progressive decrease of IL-1β but not of TNF until the 30th day after AR induction [50]. In the present study, TNF, IL-1β and IL-6 were quantified only 30 days after RA induction, probably after the disease establishment phase, justifying the absence of IL-1β and IL-6 at detectable serum levels in AR animals, but only locally in the synovial fluid of these animals. THA and ROL, or their combination, decreases IL-1β but did not affect IL-6 in synovial fluid, showing that the process should be limited to an acute phase of inflammation and persists refractory to drugs in RA.

Increased TNF levels is a hallmark of the initial and perpetuation phases of RA [3,5]. Although, RUP and PTX presented a tendency, only THA and ROL significantly decreased TNF plasma levels. In common, both drugs inhibit PDE4, a family of enzymes particularly abundant in neutrophils, T lymphocytes, macrophages and eosinophils [15,17], important sources of TNF [2]. In these cells, the PDE4 inhibitors reduced the synthesis and release of pro-inflammatory mediators, cytokines and reactive oxygen species [15]. Thus, we can hypothesize that inhibition of TNF synthesis, due to the inhibition of PDE4 in its cellular sources, promotes a consistent antiarthritic effect, whereas this effect is absent when the inhibition of PDEs is less specific and/or the inhibition of TNF occurs in cell types of minor relevance to the arthritic process. In fact, novel PDE4 inhibitors, such as cilomilast, CHF 6001, GSK256066 and MK0952 were approved for use in various autoimmune diseases such as, atopic dermatitis, psoriatic arthritis, psoriasis, systemic lupus erythematosus, vasculitis, systemic sclerosis, multiple sclerosis and inflammatory bowel disease [51]. Studies assessing the risks of using biological anti-TNF drugs consistently show an association with a higher risk of severe skin, tissue and joint damage and opportunistic infections compared to patients treated with disease modifying antirheumatic drugs [5], synthetic drugs as THA and ROL. Another advantage of the use of THA and ROL is the great amount of information regarding contraindications and severity of adverse effects of these drugs, which provide a greater control and safety for prescription.

The data presented in the present work indicate that the use of pharmacologic drugs that inhibits TNF through the inhibition of the PDE4 enzymes are promising but should be considered carefully, considering the specificities of each patient. In this sense, for patients with liver impairment the treatment with THA alone would be preferred as it restores the levels of both AST and ALT. On the other hand, the combined treatment of ROL+THA may be an effective alternative for patients who have other specific needs, such as obesity or overweight.

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## Author Contributions

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published.

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