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Fenofibrate vs pioglitazone: Comparative study of the anti-arthritic potencies of a PPAR-alpha and a PPAR-gamma agonists in rat adjuvant-induced arthritis

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Abstract.

BACKGROUND: Rheumatoid arthritis is characterized by synovial hyperplasia, inflammatory infiltration, cartilage destruction and juxta-articular as well as generalized bone demineralization. Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily which behave as ligand-activated transcription factors in response to endogenous fatty acids and eicosanoids or isotype selective synthetic compounds as fibrates and thiazolidinediones. Beyond their key role in lipid metabolism, increased evidence has shown a role of the three isotypes in inflammatory modulation. We and others demonstrated previously that PPAR-gamma agonists reduced the severity of experimental polyarthritis and the overall inflammatory-induced bone loss.

OBJECTIVE: To compare the anti-arthritic potencies of a PPAR-alpha agonist (fenofibrate, a lipid lowering drug) and a PPAR-gamma ligand (pioglitazone, formerly used as an antidiabetic drug) in rat adjuvant-induced arthritis.

METHODS: Male Lewis rats were sensitized by an intra-dermal injection of 1 mg complete Freund's adjuvant at the basis of the tail and were treated orally for 21 days with fenofibrate 100 mg/Kg/day (FENO) or pioglitazone 30 mg/Kg/day (PIO), or with vehicle only. Arthritis severity was evaluated by clinical observations (oedema, clinical score, body weight). Global and femoral bone mineral density (BMD), femoral bone mineral content (BMC) were measured by dual-energy X-ray absorptiometry (DEXA) before sensitization and at day 20. Synovial mRNA levels of IL-1beta and IL-6 were determined by real-time RT-PCR.

RESULTS: Administration of fenofibrate (100mg/kg/d) and pioglitazone at 30 mg/kg/d significantly reduced hindpaw oedema and arthritis score. Treatment with fenofibrate exerted a better effect on clinical scoring. DEXA analysis revealed that pioglitazone and fenofibrate treatment to a greater extent, reduced inflammatory-bone loss and increased BMD versus vehicle-treated rats. Finally, we demonstrated that both agonists decreased synovial expression of IL-1beta and IL-6.

CONCLUSION: Pioglitazone and fenofibrate decreased arthritis severity in adjuvant-induced arthritis. Both agonists partially protected animals from inflammatory induced-bone loss.

Keywords: Adjuvant arthritis, PPARs, pioglitazone, fénofibrate, bone

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1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily, which behave as ligand-activated transcription factors in response to endogenous fatty acids and eicosanoids or isotype selective synthetic compounds. Among the three characterized isotypes, PPAR-α is expressed essentially in tissues contributing actively to the catabolism of fatty acids and contributes to the control of inflammation. PPAR-γ is highly expressed in adipose tissue, where it has a pivotal role in adipocyte differentiation and lipid storage and is also a prominent player in inflammation control [1,2] demonstrated that all PPARs are expressed in both human and rodent osteoblasts and osteoclasts, supporting a role for the PPARs in the regulation of bone metabolism, although the significance of PPAR-α and its agonists in bone metabolism remains poorly elucidated. More is known about the role of PPAR-γ which agonists promote adipocyte differentiation preferentially over osteoblast differentiation [3].

Because of their metabolic actions, PPAR-α and PPAR-γ have become major drug targets [4]. Fenofibrate belongs to a class of fibrates, which are well-known PPAR-α agonists, and are widely used for treatment of hypercholesterolemia and hypertriglyceridemia. Thiazolidinediones are synthetic PPAR-γ agonists used for treatment of type 2 diabetes mellitus.

Experimental model of Adjuvant-Induced Arthritis (AIA) in rats reproduces major features upcoming in rheumatoid arthritis (RA). Its relevance to the pathogenesis of RA is further supported by the demonstration that pro-inflammatory cytokines are highly expressed in the developing arthritic process and by the reproduction of most bone changes found in RA [5], including inflammatory bone loss, which has been linked to an increased risk of fracture. We and others demonstrated previously that PPAR-gamma agonists reduced the severity of experimental polyarthritis and the overall inflammatory-induced bone loss. In the present study, we compare the effect of a PPAR-alpha agonist (fenofibrate) and a PPAR-gamma ligand (pioglitazone) on arthritis development and secondary bone loss.

2. Material and methods

2.1. Animals

Twenty-five inbred male Lewis rats (Charles River, L'Arbresle, France) weighing 150–175 g were acclimated for 1 week in the laboratory before use. Animals were housed in groups of three or four in solid-bottomed plastic cages with access to tap water and standard rodent pelleted chow (Scientific Animal Food & Engineering A04, Villemoisson-sur-Orge, France) *ad libitum*. Room temperature was set at 23 $\pm$ 1°C and animals were subjected to a 12-hour light cycle. All experiments were performed in accordance with national animal care guidelines and were pre-approved by a local ethics committee. Arthritis sensitization, BMD measurements and blood sampling at necropsy were performed under general anaesthesia with volatile anaesthetics (AErrane™; Baxter SA, Maurepas, France).

2.2. Induction of arthritis and treatment regimen

Arthritis was induced according to the protocol we have previously described [6]. Animals were randomly assigned to one of the following treatment groups: arthritic vehicle-treated controls (AIA + vehicle) arthritic rats treated with 30 mg/kg/day of pioglitazone (AIA + PIO). Naive animals served as controls (vehicle-treated controls) or arthritic rats treated with 100 mg/kg/day of fenofibrate (AIA +

FENO). Pioglitazone or fenofibrate were given orally from the day of sensitization until necropsy (day 21) under a volume of 1 ml/100 g body-weight. Treatment was prepared daily from marketed pills of Actos™ (Takeda, Puteaux, France) or Fénofibrate BIOGARAN® (Biogran, Colombes, France) as a suspension in 0.5% carboxymethylcellulose (CMC). Naive rats and arthritic controls received CMC only.

2.3. Assessment of arthritis

2.3.1. Body-weight

Total body-weight was recorded every other day from day 0 (D0) to day 21 (D21).

2.3.2. Arthritic score

Animals were scored regularly until day 21 (D21) by 2 investigators who were blind to the treatment. Each paw was graded according to the severity and extent of erythema and swelling of peri-articular soft tissues, and the enlargement and distortion of the joints [7]. Clinical score ranged from 0 (no sign) to 4 (severe lesions), yielding to a maximum score of 16 per animal.

2.3.3. Hindpaw œdema

Swelling of both hindpaws was measured regularly until day 21 by plethysmography. Briefly, hindpaw volume was measured up to the skin–coat junction of the rear footpad through the displacement of an equivalent volume of water in a plethysmometer 7150 (Apelex, France). At the indicated times, paw volume was compared to the basal level (D0) and œdema was expressed as changes in ml.

2.4. Bone mineral density measurements

BMD was determined *in vivo* by dual-energy X-ray absorptiometry (DEXA) using a model QDR-4500A densitometer (Hologic Inc., Waltham, MA, USA) with a small-animal module. Rats were anesthetized as mentioned above, placed in a supine decubitus position with abduction of the four limbs and scanned both the day before arthritis induction (D1) and the day before necropsy (D20). Each animal was scanned five times consecutively after repositioning, BMD measurement being expressed as mean ± SD for a single time point. BMD (g/cm^2) and bone mineral content (BMC, g) were determined on the whole body (total BMC), each measurement being performed by the same investigator who was blind to the treatment. Data were expressed as changes in BMC or BMD over study duration, each animal being used as its proper control. Internal variations of repeated measures of total rat BMD have been determined to be 1.5–2%.

Bone mineral content (BMC) and density (BMD) was determined *in vivo* by dual-energy X-ray absorptiometry (DEXA) with a densitometer QDR-4500A (Hologic Inc., MA, USA) equipped with a small-animal module. Rats were anaesthetized as mentioned above and scanned as previously described [6]. Data were expressed as changes in BMD over the study duration, each animal being used as its own control.

2.5. RNA isolation and real-time reverse transcription – polymerase chain reaction (RT-PCR)

Synovial mRNA levels were determined using SYBR Green®-based quantitative PCR. Total RNA was isolated using RNeasyplus mini kit® (Qiagen, France), which allows total removal of genomic DNA with an on-column DNA elimination step. Two hundred ng of total RNA were reverse-transcribed for 90 minutes at 37°C in a 20 µl reaction mixture containing 2.5 mM dNTP, 5 µM random hexamer

primers, 1.5 mM $MgCl_2$ and 200U Moloney Murine Leukemia Virus reverse transcriptase (Invitrogen) in a Mastercycler gradient thermocycler (Eppendorf, France).

Real time-PCR was performed using Step One Plus (Applied Biosystems) technology with set of specific primers (0.5 μM each; for sequence see Koufany et al. [6]) and iTAQ SYBRgreen master mix system (Biorad) at concentrations provided by the manufacturer. Melting curve was performed to determine the melting temperature of the specific PCR products and after amplification product size was checked on a 1% agarose gel stained with ethidium bromide. Each run included positive and negative reaction controls. The mRNA level of the gene of interest and of S29, chosen as housekeeping gene, was determined in parallel for each sample. Quantification was determined using the $\Delta\Delta cT$ methods and results were expressed as fold change over control.

2.6. Statistical analysis

Data are expressed as mean ± SEM. Arthritis score and histological grading were analysed with the Kruskall–Wallis test, using StatView™ version 5.0 software (SAS Institute Inc., Cary, NC, USA). All other data were compared by analysis of variance (ANOVA) followed by Bonferroni *post-hoc* test. Differences were considered significant at $P < 0.05$ ($^{*}P < 0.05$ compared with vehicle-treated controls (Control); $^{\#}P < 0.05$ compared with arthritic vehicle-treated rats (AIA)).

3. Results

3.1. Effect of pioglitazone and fenofibrate on arthritis severity

Arthritis occurred in all animals sensitized with complete Freund's adjuvant. Treatment with pioglitazone or fenofibrate did not reduce arthritis incidence (data not shown). As shown in Fig. 1(a), arthritis became obvious by day 13 and was maximal around day 20 after sensitization. At necropsy, the arthritic score averaged 13 in vehicle-treated animals, reflecting a severe arthritis, confirmed by ankle joint histological analysis (data not shown). Analysis of paw volume by plethysmometry revealed a bilateral paw swelling at day 13 with a maximum around day 17. Pioglitazone and fenofibrate treatment reduced both clinical score and oedema to a higher extent with fenofibrate.

Body-weight of naive animals increased gradually with a mean gain of approximately 5 g/day over study duration (Fig. 1(c)). In all arthritic rats, body-weight peaked at day 9, then decreased when arthritis settled. The rate of changes in body-weight was similar in arthritic controls and in rats treated with pioglitazone. The loss in body-weight gain was reduced from D13 to D20 in arthritic animals receiving 100 mg/kg/d of fenofibrate.

3.2. Effect of pioglitazone and fenofibrate on arthritis-induced bone changes

Changes in BMC and BMD were evaluated by DEXA on the whole body and on one region of interest (left femur). BMD increased over study duration globally and in the region of interest of vehicle-treated controls (data not shown). Arthritic animals showed a limited progression of BMD, due to the development of the disease. Fenofibrate treatment induced global and femoral BMD. A limited decrease in BMC (Fig. 2(c)) was observed in arthritic AIA controls. This loss in BMC was prevented in arthritic animals treated with 30 mg/kg/d of pioglitazone or with 100 mg/kg/d of fenofibrate (Fig. 2(c)).

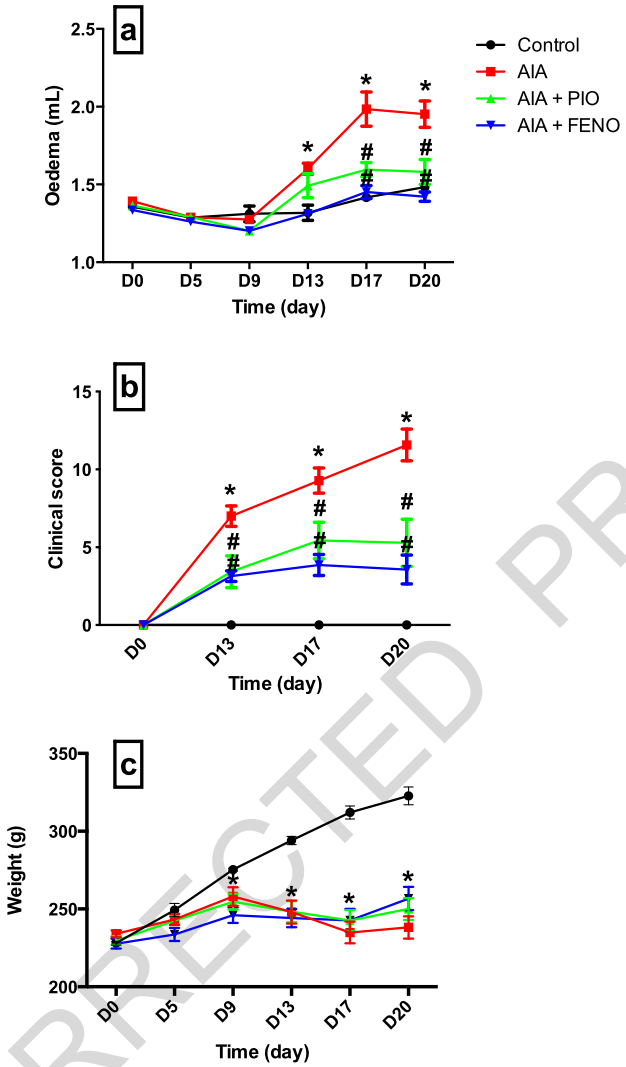

Fig. 1. Oedema (a), arthritic score (b), body-weight (c). Lewis male rats were sensitized subcutaneously at the basis of the tail with 1 mg of *M. tuberculosis*. Animals were treated daily with 100 mg/kg of FENO ($n = 6$) or 30 mg/kg of PIO ($n = 6$) by oral administration. Arthritic (AIA, $n = 6$) and healthy controls ($n = 6$) were given CMC 0.5% alone. Data are expressed as mean $\pm$ SEM. $* \; p < 0.05$ vs control and $\# \; p < 0.05$ vs AIA groups. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/BME-140977.)

3.3. Effect of PPAR agonists on cytokines expression

Synovial mRNA expression of IL-1beta, IL-6 and IL-17 was determined by RT-PCR. IL-17 was barely detectable in our experimental conditions. Interleukin-1beta was overexpressed in arthritic animals compared to healthy controls. Treatment with both agonists significantly reduced IL-1beta expression (Fig. 3). The same observation was made with IL-6, agonists of the both alpha and gamma PPAR isotypes totally suppressed IL-6 induction in arthritic treated animals.

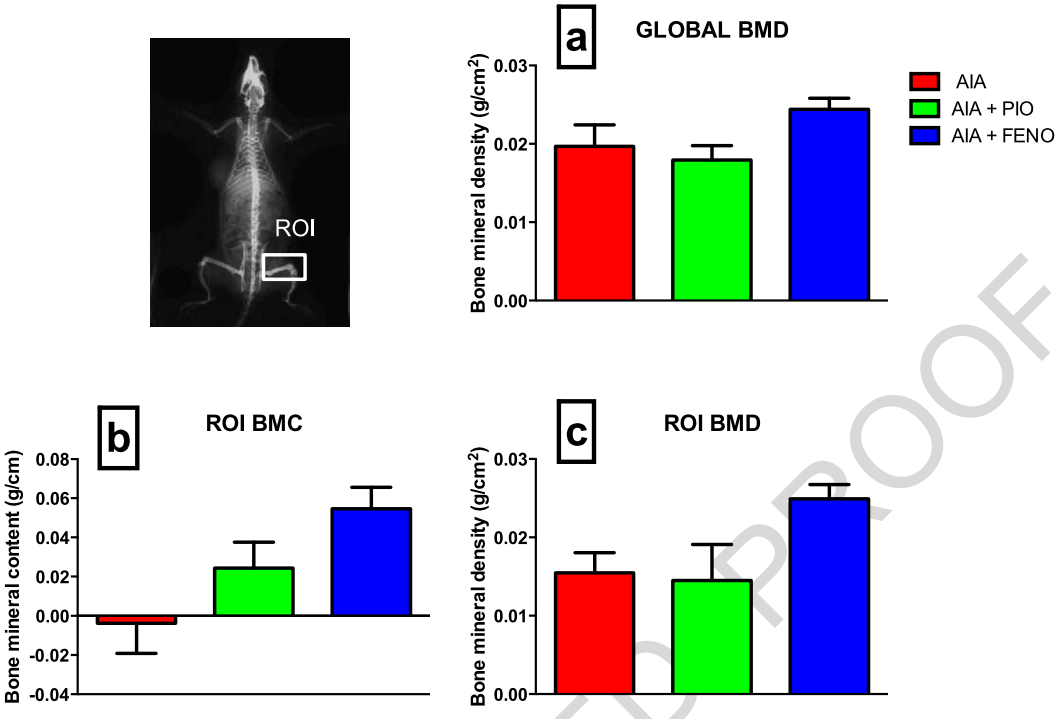

Fig. 2. Effect of fenofibrate (FENO) and pioglitazone (PIO) treatment on bone mineral density in adjuvant-induced arthritic rats. Global bone mineral density at day 20 (a), femoral bone density at day 20 (b), femoral bone content (day 20–day 0) (c). Male Lewis rats were sensitized subcutaneously on the basis of the tail 1 mg M. tuberculosis. Animals were treated daily with FENO 100 mg/kg ($n = 6$) or PIO 30 mg/kg ($n = 6$) by oral administration. Arthritic (AIA, $n = 6$) and normal controls ($n = 6$) were given CMC 0.5% alone. Data are expressed as mean ± SEM. # $p < 0.05$ vs AIA controls. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/BME-140977.)

4. Discussion

In our experimental model, fenofibrate at 100 mg/kg/day and pioglitazone at 30 mg/kg/day decreased several aspects of arthritis severity as shown by partial inhibition of paw edema and reduction of arthritic score. The anti inflammatory potency of fenofibrate and pioglitazone extended the general meaning that inflammation was a target for PPAR-α and PPAR-γ agonists as reported by previous studies [8–12].

We demonstrated that both agonists reduced the expression in arthritic synovial of two key cytokines involved in arthritic process, IL-6 and IL-1β, this finding being consistent with the report of their decrease by a PPAR-γ agonist in joints of mice with established collagen-induced arthritis [9]. In fenofibrate treatment, pro-inflammatory cytokines inhibition could be attributed to the ability of PPAR-α agonists to inhibit the NF-κB pathway in arthritic tissues [8].

Focal erosions or generalized osteopenia, are a very active topics in RA. In our study, animals with AIA reproduced this important bone resorption administration of fenofibrate or pioglitazone prevented arthritis-induced bone loss. We already demonstrated a bone protective effect of pioglitazone in adjuvant-induced arthritis. Indeed, pioglitazone decreased inflammatory-bone destruction and reduced bone micro-architecture in AIA rats by controlling circulating and local expression of IL-17 with a subsequent decrease of the (RANKL/OPG) ratio [13]. Of interest, three recent studies have demonstrated the efficacy of pioglitazone alone or in combination with methotrexate in RA patients [14–16].

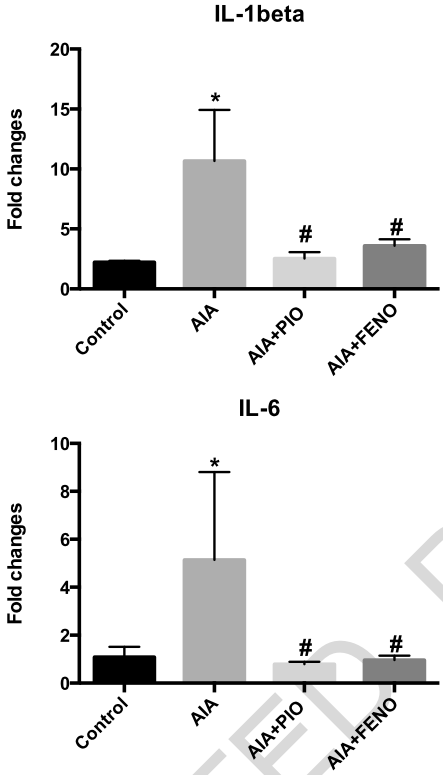

Fig. 3. Effect of fenofibrate (FENO) and pioglitazone (PIO) treatment on IL-1beta and IL-6 synovial mRNA expression. Male Lewis rats were sensitized subcutaneously on the basis of the tail 1 mg *M. tuberculosis*. Animals were treated daily with FENO 100 mg/kg ($n = 6$) or PIO 30 mg/kg ($n = 6$) by oral administration. Arthritic (AIA, $n = 6$) and normal controls ($n = 6$) were given CMC 0.5% alone. Data are expressed as mean $\pm$ SEM. # $p < 0.05$ vs AIA controls.

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