Up-Regulation of the Inflammatory Response by Ovariectomy in Collagen-Induced Arthritis. Effects of Tin Protoporphyrin IX

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Abstract—We have studied the influence of ovariectomy on the inflammatory response and bone metabolism on CIA as a model of postmenopausal arthritis as well as the effects of tin protoporphyrin IX (SnPP), a heme oxygenase inhibitor. Ovariectomy in non-arthritic mice produced increased serum PGD2 levels and up-regulated the expression of COX-2, h-PGDS, l-PGDS, and HO-1 in the joints. In CIA, ovariectomy potentiated the inflammatory response with higher levels of serum IL-6 and MMP-3, local PGD2 and MMP-3 as well as trabecular bone erosion. In OVX-CIA, SnPP decreased the serum levels of IL-6, MMP-3, and PGD2; down-regulated TNFα, COX-2, hPGDS, PGD2, PGE2, and MMP-3 in joint tissues; and also decreased focal bone loss in the inflamed joint. Ovariectomy up-regulates inflammatory mediators in non-arthritic and in arthritic animals. In the OVX-CIA model, SnPP exerts anti-inflammatory effects which are not associated with the prevention of systemic bone loss.

KEY WORDS: ovariectomy; collagen-induced arthritis; bone metabolism; prostaglandin D2; tin protoporphyrin IX.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease characterized by persistent synovial inflammation as well as articular cartilage and bone destruction. In particular, focal bone erosion, periarthritis osteopenia adjacent to inflamed joints, and generalized osteoporosis have been described, resulting in an important disability [1].

The incidence of RA and joint destruction is greater in women compared with men and can be related to menopause [2, 3]. A decrease in estrogen levels following menopause can have a profound impact on the development and progression of autoimmune diseases. It is known that hormone replacement therapy or estrogen administration exerts inhibitory effects on the inflammatory and bone changes of RA, but they are associated with important side effects [4, 5].

The mouse collagen-induced arthritis (CIA) has been extensively used as a model of RA. Recently, induction of arthritis by type II collagen in ovariectomized DBA/1 mice has been proposed as a model of postmenopausal RA where the loss of estrogen and inflammation contribute equally to osteoporosis [6].

Heme oxygenase-1 (HO-1) is induced in many cells by oxidative stress and a wide range of stimuli. Induction of this protein may exert antioxidant effects

ABBREVIATIONS: AP, Alkaline phosphatase; CII, Type II collagen; CIA, Collagen-induced arthritis; COMP, Cartilage oligomeric matrix protein; COX-2, Cyclooxygenase-2; ELISA, Enzyme-linked immunos assay; HO-1, Heme oxygenase-1; IL, Interleukin; h-PGDS, Hematopoietic prostaglandin D synthase; l-PGDS, Lipocalin-prostaglandin D synthase; MMP, Matrix metalloproteinase; NA, Naive group; OVX, Ovariectomized group; OVX-CIA, Ovariectomized arthritis group; RA, Rheumatoid arthritis; RANKL, Receptor activator of nuclear factor-κB ligand; SnPP, Tin protoporphyrin IX; TRAP-5b, Tartrate-resistant acid phosphatase 5b; TNFα, Tumor necrosis factor-α
(reviewed in [7]) and may regulate osteoclastogenesis [8]. In osteoblasts, HO-1 may protect against tumor necrosis factor-α (TNFα)-induced apoptosis [9], but it may also inhibit the maturation of these cells [10]. Although tin protoporphyrin IX (SnPP), an inhibitor of HO activity, exerts anti-inflammatory effects on mouse CIA [11], whether this agent is active on bone metabolism or the arthritic process in estrogen deficiency is unknown. The current studies were performed in ovariectomized mice with CIA to further characterize this animal model and its possible use in the search of novel pharmacological treatments for inflammatory bone loss. Because SnPP can exert anti-inflammatory effects, we examined whether this treatment may be active on the inflammatory and bone metabolic processes involved in experimental postmenopausal arthritis.

MATERIALS AND METHODS

Animals

Female DBA-1/J mice between 10 and 12 weeks of age were obtained from Janvier (Le Genest St Isle, France). Water and food were provided ad libitum. All studies were performed in accordance with European Union regulations for the handling and use of laboratory animals. The protocols were approved by the institutional animal care and use committee (University of Valencia, Spain).

Ovariectomy

A total of 30 mice were ovariectomized. Ovaries were removed through a midline incision of the skin and flank incisions of the peritoneum. The skin incision was then closed with metallic clips. Surgery was performed after the mice were anesthetized with isoflurane and treated with butorfanol (2 mg/kg, s.c.).

Induction of CIA

Two weeks after surgery (day 0), ovariectomized and non-ovariectomized DBA-1/J mice were immunized at the base of the tail with 100 mg of bovine type II collagen (CII) prepared as previously described [12] and diluted in 0.05 M acetic acid to a concentration of 2 mg/ml. This was emulsified in equal volumes of Freund’s complete adjuvant (2 mg/ml Mycobacterium tuberculosis, strain H37Ra; Difco, Detroit, MI, USA). On day 21, mice received an intraperitoneal booster injection of 100 mg of CII dissolved in phosphate buffered saline. Mice were considered to have arthritis when significant changes in redness and/or swelling were noted in the digits or in other parts of the paws. Joint inflammation was scored visually in each paw, using a scale of 0–2 where 0=uninflamed, 1=mild, 1.5=marked, and 2=severe. Scoring was performed by two independent observers without knowledge of the experimental groups.

Treatment Groups

Animals with a minimum score of 1 on day 22 were then randomized into different CIA groups. Mice were divided into five groups according to ovariectomy, CIA, and treatment administration: NA, naïve group (n=5); OVX, ovariectomized group (n=6); CIA, arthritic group (non-ovariectomized) (n=8); OVX-CIA, ovariectomized arthritic group (n=12); and OVX-CIA+SnPP, ovariectomized arthritic mice treated with SnPP (n=12). SnPP (12 mg/kg of body weight, intraperitoneally once a day) was administered from day 22 to day 42 after immunization. The dose was selected in preliminary experiments. NA, OVX, and CIA groups were administered the vehicle (200 μl of 1% dimethyl sulfoxide in saline) in the same way. On day 43, blood samples were taken by retro-orbital puncture and animals were killed by cervical dislocation.

Histological Analysis

After mice were killed, whole knee joints were removed and fixed in 10% formalin. After decalcification in 5% formic acid, the specimens were processed for paraffin embedding. Tissue sections (7 μm) were stained with hematoxylin and eosin or safranin O. Infiltration of cells in the synovial cavity (exudate) and synovial tissue (infiltrate) was scored on a scale of 0–3. Proteoglycan depletion was determined using safranin O staining. The loss of proteoglycan, chondrocyte death, bone erosion, and cartilage erosion were scored separately on a scale of 0–3 [12]. Two observers without knowledge of the experimental group performed the scoring.

Immunohistochemistry

After mice were killed, whole knee joints were removed and fixed in 10% formalin. After decalcification in 10% EDTA, the specimens were processed for paraffin embedding. Tissue sections (7 μm) were treated
with 2% H2O2 for 10 min at room temperature; sections were incubated for 2 h with rabbit antibodies against cyclooxygenase-2 (COX-2), hematopoietic-prostaglandin D synthase (h-PGDS), lipocalin-prostaglandin D synthase (l-PGDS) (Cayman Chemical, Ann Arbor, MI, USA), and HO-1 (Stressgene, Victoria, BC, Canada). Rabbit Ig antibody (Dako, Glostrup, Denmark) was used to perform negative controls. After rinsing, sections were incubated with the correspondent secondary biotinylated antibody (Dako) and processed using streptavidin–horseradish peroxidase (Dako). Peroxidase staining was developed with diaminobenzidine (Sigma). Counterstaining was performed with hematoxylin. Positive cells were counted in five random high-power fields by two independent observers.

**Enzyme-Linked Immunosorbent Assay, Luminex, and Radioimmunoassay**

Serum were used for the determination by enzyme-linked immunoassay (ELISA) of anti-CII IgG, with sensitivity of 0.375 ng/ml, cartilage oligomeric matrix protein (COMP) with sensitivity of 0.2 U/l (MD Biosciences, Zürich, Switzerland), PGD2 with sensitivity of 0.2 ng/ml (Cayman Chemical), matrix metalloproteinase-3 (MMP-3) with sensitivity of 10 pg/ml (RayBiotech, Inc., Norcross, GA, USA), and tartrate-resistant acid phosphatase 5b (TRAP-5b) with sensitivity of 0.1 U/ml (IDS, Paris, France). Serum levels of interleukin(IL)-6 and IL-17 were determined by luminex, with sensitivity of 1.8 pg/ml and 0.5 pg/ml, respectively (Millipore Corporation, Billerica, MA, USA). Alkaline phosphatase (ALP) levels in serum were determined as previously described [13]. Hind paws were amputated above the ankle and homogenized in 1 ml of 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, pH 7.4. Supernatants were used for the determination of PGE2 by radioimmunoassay [14], PGD2 and MMP-3 by ELISA as above, and IL-1β and TNFα using ELISA kits with sensitivity of 3 and 5.1 pg/ml, respectively (R&D Systems, Abingdon, UK).

**X-Ray Microcomputed Tomography**

Three-dimensional trabecular microarchitecture was analyzed by μCT. The region of cancellous bone from
proximal diaphysis of the tibia, between the subchondral plate and the growth plate, was analyzed without further sample preparation with a SkyScan 1172 μCT equipment (SkyScan NV, Aartselaar, Belgium). Samples were imaged with an X-ray tube voltage of 100 kV, current of 100 μA, and at a scanning voxel size of 6.0 μm. The scanning angular rotation was 185° and the angular increment was 0.4°. Datasets were reconstructed using a modified Feldkamp algorithm [15] and segmented into binary images using adaptive local thresholding. Cancellous bone regions were obtained by free drawing regions of interest excluding cortical bone and analyzed using the commercial software provided with the equipment (SkyScan CT-Analyzer software). Morphometric indices of trabecular bone region were determined from the microtomographic datasets (integrated over a volume of interest) using direct 3D morphometry. Tissue volume (TV, mm³), bone volume (BV, mm³), and bone surface (BS, mm²) were calculated by marching cubes method. Bone volume fraction (BV/TV, %), bone surface density (BS/TV, mm⁻¹), and bone-specific surface (BS/BV, mm⁻¹) were directly calculated. Trabecular thickness

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**Fig. 2.** Histological analysis of the frontal sections of knee joints on day 43. a – E: hematoxylin and eosin staining, F–J: safranin O staining. A, F: NA naïve mouse group; B, G: OVX ovariectomized group. C, H: CIA non-ovariectomized arthritic group. D, I: OVX+CIA ovariectomized arthritic group. E, J: OVX+CIA+SnPP ovariectomized arthritic mice treated with SnPP (12 mg/kg/day from days 22 to 42 after immunization). Original magnification ×100. b Synovial infiltrate, synovial exudate, cartilage erosion, chondrocyte death, bone erosion, and proteoglycan depletion were scored on a scale of 0–3. Data represent mean±SEM. ++P<0.01 versus NA; +++P<0.01 versus OVX; *P<0.05 versus OVX+CIA.
(Tb.Th, mm), trabecular spacing (Tb.Sp, mm), and trabecular number (Tb.N, mm\(^{-1}\)) were measured directly on 3D images using methods previously described [16, 17].

**Statistical Analysis**

The results are presented as mean±standard error of the mean (SEM). The level of statistical significance was determined by the two-tailed unpaired Student’s \(t\) test and the Mann–Whitney test for score analyses.

**RESULTS**

As shown in Fig. 1a and b, ovariectomy increased the inflammatory manifestations of disease leading to a clinical score for OVX-CIA animals significantly higher than that of CIA mice on day 43. SnPP treatment was begun on day 22 after induction, when arthritis was evident. After 3 days of treatment, the clinical score was already reduced with respect to the control OVX-CIA animals (\(P<0.01\)), and this anti-inflammatory effect of SnPP was observed at all time points until day 43. At the end of the experiment, the weight of OVX animals had increased with respect to the NA group (Fig. 1c), although it did not reach statistical significance. In contrast, arthritic animals (CIA and OVX-CIA groups) exhibited weight loss in a very significant manner (\(P<0.01\) with respect to NA and OVX, respectively), and SnPP treatment counteracted this negative effect of arthritis (OVX-CIA+SnPP, \(P<0.01\) with respect to OVX-CIA).

The results of histological evaluation of mouse knees are summarized in Fig. 2. The arthritic groups CIA and OVX-CIA showed a high level of inflammatory cellular infiltration and cellular exudate into the joint.
space, as well as proteoglycan depletion, cartilage erosion, chondrocyte death, and bone erosion, with no significant differences between both groups. SnPP administration resulted in a significant reduction in the last three parameters.

The serum levels of anti-collagen II antibodies were significantly increased in arthritic animals, although they were not modified by ovariectomy (Fig. 3). SnPP did not exert any effect on serum anti-collagen II antibody levels. The marker of cartilage degradation COMP was not significantly affected by ovariectomy and strongly increased in arthritic mice. It is interesting to note that animals treated with SnPP showed significant reductions in serum COMP levels.

Figure 4a and b shows the serum concentrations of the bone synthesis marker ALP and the bone resorption marker TRAP-5b, which were not significantly affected by ovariectomy. In contrast, the arthritic process in CIA and OVX-CIA groups led to important changes, with ALP reductions and TRAP-5b increases. None of these markers were significantly modified by SnPP treatment. The results of three-dimensional trabecular microarchitecture analysis by μCT (Fig. 4c and Table 1) indicate that ovariectomy resulted in a reduction in bone volume fraction, bone surface density, trabecular thickness and trabecular number, and increased bone specific surface. Arthritis caused more important effects on these parameters, as observed in the CIA group, whereas ovariectomy aggravated the deleterious effects of CIA on trabecular bone. Treatment of animals with SnPP decreased bone volume fraction and increased bone surface density, bone-specific surface, and trabecular number.

Serum IL-6 and MMP-3 were significantly increased in CIA and OVX-CIA versus NA and OVX, respectively (Fig. 5a, c). In addition, OVX-CIA animals showed IL-6 and MMP-3 levels higher than those in the CIA group. In OVX-CIA mice, SnPP therapy normalized serum IL-6 and MMP-3 to levels that were significantly lower than in OVX-CIA control animals. In contrast, serum of IL-17 was not modified by ovariectomy or arthritis induction (Fig. 5b) and IL-1β and TNFα were not detectable (data not shown). Figure 5d shows that OVX, CIA, and OVX-CIA animals showed significant increases in serum PGE2 levels, whereas treatment with SnPP caused a significant reduction in this prostanoid.

IL-1β and TNFα were also measured in paw homogenates (Fig. 6a, b). Arthritic mice (CIA and OVX+CIA) showed high levels of these cytokines. The animals in the OVX-CIA+SnPP group exhibited a significant reduction in TNFα with respect to the OVX-CIA control. MMP-3 in paw homogenates was significantly increased in CIA and OVX-CIA versus NA and OVX, respectively (Fig. 6c). In addition, OVX-CIA animals showed MMP-3 levels higher than those in the CIA group, and SnPP therapy decreased MMP-3 to levels that were significantly lower than in OVX-CIA control animals (Fig. 6c). As PGE2 was hardly detected in serum (data not shown), we measured local PGE2 levels using paw homogenates. Figure 6d shows that this prostanoid significantly increased in arthritic animals (CIA and OVX+CIA groups) and SnPP treatment reduced local PGE2. Although PGD2 levels did not change in OVX or CIA mice, they significantly increased in OVX-CIA animals and were inhibited by SnPP (Fig. 6e).

Table 1. Trabecular Bone Analysis

<table>
<thead>
<tr>
<th></th>
<th>BV/TV (%)</th>
<th>BS/BV (mm⁻¹)</th>
<th>BS/TV (mm⁻¹)</th>
<th>Tb.Th (mm)</th>
<th>Tb.Sp (mm)</th>
<th>Tb.N (mm⁻¹)</th>
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<td>NA</td>
<td>20.8601</td>
<td>76.6497</td>
<td>15.9892</td>
<td>0.0539</td>
<td>0.2189</td>
<td>3.8721</td>
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<td>OVX</td>
<td>18.287</td>
<td>84.3344</td>
<td>15.4222</td>
<td>0.0492</td>
<td>0.2182</td>
<td>3.7141</td>
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<tr>
<td>CIA</td>
<td>12.624</td>
<td>97.9927</td>
<td>12.3706</td>
<td>0.0473</td>
<td>0.2318</td>
<td>2.6718</td>
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<tr>
<td>OVX-CIA</td>
<td>8.4794</td>
<td>97.6979</td>
<td>8.2842</td>
<td>0.0475</td>
<td>0.3018</td>
<td>1.7857</td>
</tr>
<tr>
<td>OVX-CIA+SnPP</td>
<td>7.7802</td>
<td>108.7833</td>
<td>8.4635</td>
<td>0.0422</td>
<td>0.3965</td>
<td>1.8439</td>
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The region of cancellous bone from proximal diaphysis of the tibia, between the subchondral plate and the growth plate, was analyzed by μCT. Results from a representative experiment
Ovariectomy also increased the expression of h-PGDS (OVX, 121.6±6.1, P<0.01 versus NA, 37.0±7.5) and l-PGDS (OVX, 81.8±4.2, P<0.05 versus NA, 54.7±10.4) in non-arthritic mice as well as in arthritic animals (P<0.01 versus OVX-CIA, 72.5±17.0 for h-PGDS, and P<0.05 versus OVX-CIA, 39.3±15.0 for l-PGDS). The treatment of OVX-CIA animals with SnPP decreased the number of cells positive for h-PGDS (17.7±12.2, P<0.05) versus OVX-CIA+SnPP, 62.0±14.3, P<0.05 versus OVX+CIA).

DISCUSSION

The present study examined the effects of ovariectomy on articular structure, protein expression, production of inflammatory mediators, and bone turnover in DBA-1/J mice. We have found an increased expression of COX-2, h-PGDS, l-PGDS, and HO-1 in the articular sections of the OVX group in ovariectomized non-arthritic mice in comparison with normal animals. The up-regulation of enzymes involved in prostanoid synthesis resulted in increased serum levels of PGD2. Nevertheless, no significant changes in clinical nor histological scores and no effects on trabecular bone were observed.

In immune arthritis, cartilage destruction is accompanied by focal bone erosion in affected joints which is dependent on increased osteoclast activity [18]. The systemic disease is reflected by increased serum concentrations of inflammatory mediators and bone turnover markers [19]. We have shown that ovariectomy potentiates the systemic inflammatory response in arthritis, with higher levels of IL-6, MMP-3, and PGD2. In addition, local joint inflammation was also enhanced by ovariectomy leading to a higher arthritic score and PGE2 content. Nevertheless, we did not observe any significant modification by ovariectomy of the immune response, histological score, or serum COMP levels. In this model of postmenopausal arthritis, SnPP administration after the onset of disease reduced the progression of arthritis without modification of serum levels of antibodies against collagen II. However, SnPP protected against weight loss and significantly reduced the arthritic score, although cellular infiltration was not modified. We have also observed protective effects of SnPP on cartilage as indicated by the reductions in COMP levels and histological score.
In RA, there is an increased bone turnover, especially in the resorptive phase of the periarticular trabecular bone. The mechanism of bone loss would be dependent on a negative balance in which the amount of bone formed is less than the amount of bone resorbed. Serum markers of bone metabolism indicate that CIA leads to an increase in osteoclast activity accompanied by a decrease in osteoblast activity. The consequence would be an important trabecular bone resorption as bone trabeculae were thinner and decreased in number, which was further enhanced by ovariectomy. We investigated the effects of SnPP on bone metabolism and found no significant modifications in the serum levels of bone turnover markers or trabecular structure in the animals treated with this agent, although it partially reduced focal bone erosion in the joint.

Progression of bone damage is dependent on both inflammation and osteoclast activation [20], which may be induced by soluble factors from the rheumatoid synovium [21]. Osteoclasts are markedly activated after

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**Fig. 6.** Levels of inflammatory mediators measured in paw homogenates on day 43. a IL-1β, b TNFα, c MMP-3, d PGE2, e PGD2. NA naïve mouse group, O VX ovariectomized group, CIA non-ovariectomized arthritic group, O VX-CIA ovariectomized arthritic group, O VX-CIA+SnPP ovariectomized arthritic mice treated with SnPP (12 mg/kg/day from days 22 to 42 after immunization). Data represent mean±SEM. ++P<0.01 versus NA, +++P<0.05, +++P<0.01 versus OVX, #P<0.05, ##P<0.01 versus CIA, *P<0.05, **P<0.01 versus OVX+CIA.
binding of the receptor activator of nuclear factor κB ligand (RANKL) to RANK expressed on the surface of osteoblasts and monocytic cells. Pro-inflammatory cytokines such as IL-1β, IL-6, IL-17, or TNFα up-regulate the expression of RANKL contributing to bone loss in arthritis [22]. In addition, estrogen loss results in IL-6-mediated stimulation of osteoclastogenesis, which suggests a mechanism for the increased bone resorption in postmenopausal osteoporosis [23]. The results of this study show that SnPP decreases serum IL-6 levels and joint TNFα levels. Although SnPP was effective against focal bone loss in the inflamed joint, it was unable to control the changes in systemic bone metabolism in this model of postmenopausal arthritis, and thus our results suggest that local and systemic bone erosion can be dissociated in this experimental model of postmenopausal arthritis and that down-regulation of the inflammatory response is not sufficient to control systemic bone metabolism.

The MMPs are a family of zinc-dependent endopeptidases capable of degrading all components of the extracellular matrix. MMP action is controlled at several levels including the regulation of synthesis by hormones, cytokines, and growth factors. In particular, MMP-3 is up-regulated in synovia from patients with rheumatoid arthritis [24], suggesting its contribution to the increased cartilage degradation in this disease. Recently, Seeuws et al. (2010) have demonstrated a marked association between serum MMP-3 levels and all features of CIA including inflammation, cartilage destruction, and bone erosion [25].

Estrogen withdrawal in mice is associated with increased IL-6 [23] and MMP-3 expression [26]. Our results show that ovariectomy increases the levels of MMP-3 in the arthritic joint as well as in the serum, and
the treatment with SnPP is able to significantly reduce the amount of this enzyme. These inhibitory effects on MMP-3 production can contribute to control, at least in part, the destruction of cartilage and bone.

Pro-inflammatory cytokines induce the activation of NF-kB and the transcription of a wide range of pro-inflammatory and catabolic mediators. The expression of COX-2 and the production of PGs are regulated by cytokines and participate in inflammation and destructive mechanisms in the joint [27]. Studies in COX-2-deficient mice indicate that this enzyme plays a critical role in bone resorption stimulated by 1,25 dihydroxy vitamin D and parathyroid hormone [28]. PGE2 exerts complex actions on bone metabolism with an important role in bone remodeling. Osteoclast formation and bone resorption induced by IL-1β and IL-6 are mediated by this prostanoid, although it also promotes bone formation in vitro by stimulating osteoblastic proliferation and differentiation [29, 30]. In fact, the administration of PGE2 stimulates bone formation in ovariectomized mice [31], although the anabolic effects of PGE2 administration can be counteracted by catabolic effects [32] and treatment with a selective COX-2 inhibitor reduced serum levels of a bone resorption marker but did not result in prevention of bone loss [33]. In addition, different PGE2 effects would be mediated by different receptors, as EP4 activation induces bone remodeling in vitro [34] and EP1-specific antagonism inhibits osteoclast formation induced by RANKL [35]. We have shown that SnPP down-regulates COX-2 expression and also lowers local PGE2 levels in joint tissues, which may participate in its local anti-inflammatory effects.

Little is known of the role that PGD2 plays in bone metabolism. In human osteoblasts, IL-1β induces PGD2 which acts on the DP1 receptor to decrease osteoprotegerin production and on the CRTH2 receptor to decrease RANKL [36]. This prostanoid has shown inhibitory effects on osteoclastogenesis from peripheral blood mononuclear cells in vitro [37]. Our study provides evidence for the participation of PGD2 in the inflammatory processes of CIA and OVX-CIA. In this experimental model of postmenopausal arthritis, our data indicate that SnPP is able to reduce the enhanced serum and joint levels of PGD2, which could be dependent on the down-regulation of hPGDS. These inhibitory effects on PGD2 production can contribute to control the systemic and local inflammatory responses.

HO-1 induction can exert anti-inflammatory effects in different tissues [7]. In the present study, we have shown that SnPP treatment induces the expression of HO-1 in joint tissues. Although this agent is an inhibitor of this enzyme activity, other non-specific mechanisms may be involved in the biological effects of this class of drugs [38], and there is experimental evidence suggesting the participation of HO-1 induction in the protective effects of this protoporphyrin [39]. Further studies would be needed to establish the mechanisms of action of SnPP.

We have found novel effects of ovariectomy on enzyme up-regulation in articular tissues and the production of PGD2. Our results suggest that this prostanoid is part of the systemic inflammatory response of immune arthritis. In the OVX-CIA model of postmenopausal arthritis, we have demonstrated the anti-inflammatory effects of SnPP which are dependent on the systemic and local reduction of inflammatory mediators. Our data also indicate the presence of protective effects of SnPP against cartilage destruction. Nevertheless, these anti-arthritis effects are not associated with the prevention of systemic bone loss.

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