Pharmacologic Induction of Heme Oxygenase 1 Reduces Acute Inflammatory Arthritis in Mice

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Objective. To determine the consequences of pharmacologic up-regulation of heme oxygenase 1 (HO-1), and inhibition of HO-1 by injection of an anti–HO-1 small interfering RNA (siRNA), in vivo in the acute phase of a mouse model of nonautoimmune arthritis.

Methods. In the K/BxN mouse serum transfer model, which mimics human inflammatory arthritis without lymphocyte influence, HO-1 was up-regulated by intraperitoneal injection of cobalt protoporphyrin IX (CoPP), a potent pharmacologic inducer, and was inhibited using a specific siRNA. The clinical progress of arthritis was monitored by measurement of paw thickness. Interleukin-1β (IL-1β), IL-6, tumor necrosis factor α (TNFα), serum antioxidant, and nitric oxide (NO) levels, prostaglandin E2 (PGE2) production, and matrix metalloproteinase 9 (MMP-9) activity were measured in serum. At the end of the experiments, joints were examined for immunohistopathologic changes.

Results. Intraperitoneal injection of CoPP alleviated disease symptoms, such as joint swelling, cartilage degradation, and proliferation of inflammatory tissue in joints, in the acute phase of inflammatory arthritis. The CoPP-induced expression of HO-1 in the joints and liver was associated with marked decreases in IL-1β, IL-6, and TNFα levels, PGE2 secretion, and MMP-9 activity in serum, and with a marked increase in systemic antioxidant activity. In contrast, NO production in serum and inducible NO synthase expression in chondrocytes were not affected by HO-1 induction. Specific inhibition of HO-1 by in vivo delivery of anti–HO-1 siRNA repressed the protective effects.

Conclusion. Our data provide the first evidence that pharmacologically induced up-regulation of HO-1 triggers a robust protective antiinflammatory response in a model of nonautoimmune arthritis in mice. This suggests that exogenously induced HO-1 may have potential as therapy in the acute phase of inflammatory arthritis in humans.

The recurrence and the intensity of the acute phase of joint inflammation are important factors in the destruction of adjacent cartilage and bone in inflammatory forms of human arthritis, such as acute gout and pseudogout, infectious arthritis, seronegative spondylarthritides, rheumatoid arthritis, and juvenile arthritis. Cumulative evidence indicates that systemic proinflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor α (TNFα), play a central role in the pathogenesis of this innate acute phase of joint inflammation (1–3), and synergistic inhibition of all of these cytokines would be an interesting approach to therapy in this acute inflammatory phase. Glucocorticoids inhibit the production and/or effects of all of these cytokines, but their various adverse effects, mainly in children and in patients with infectious arthritis, have prompted the search for new antagonistic molecules. Among them, IL-10 has been

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shown to inhibit the production of inflammation mediators in arthritis (4–7). Heme oxygenase 1 (HO-1), known for its cytoprotective effects against oxidative damage (ref. 8; for review, see ref. 9), has been suggested to exert antiinflammatory actions per se and also to mediate the protective effects of IL-10 against inflammation (for review, see refs. 10 and 11).

HO-1 catalyzes the rate-limiting step of heme degradation by oxidative cleavage of the α-meso carbon bridge to produce equimolar quantities of biliverdin IX, free iron, and carbon monoxide. The enzyme is induced by heme, its synthetic analog cobalt protoporphyrin IX (CoPP), and various cell stress conditions, such as hyperoxia, hypoxia, heat shock, and increased levels of endotoxin, hydrogen peroxide, oxidized lipoproteins, cytokines, ultraviolet light, and heavy metals. HO-1 expression has also been reported to be induced in vitro and in vivo by antiinflammatory molecules, such as simvastatin (12) or resveratrol (13), in human and rat smooth muscle cells, by IL-10 in lipopolysaccharide (LPS)–activated macrophages (14), by 15-deoxy-Δ12,14-prostaglandin J2 (15-deoxy-Δ12,14-PGJ2) in LPS-activated macrophages (15), and by antiarthritic gold compounds in peritoneal macrophages and U937 and Jurkat cell lines (16,17).

HO-1 overexpression or induction has been associated with antiinflammatory events. Transgenic mice that overexpress HO-1 in the lungs are protected against pulmonary inflammation (18). Intratracheal inoculation of mice with an adenovirus encoding HO-1 increases the Th2 response in macrophages (19). Corneal inflammation is less severe when HO-1 is concomitantly induced (20). IL-10 or 15-deoxy-Δ12,14-PGJ2, known inducers of HO-1, alleviate adjuvant-induced arthritis (21) and LPS-induced septic shock (14) in mice. A human patient who was deficient in the HO-1 gene exhibited a severe phenotype of general inflammation leading to death (22), and a similar observation was reported in HO-1–null mice (23,24), in which inactivation of the HO-1 gene was associated with a Th1-weighted shift in cytokine response.

HO-1 is expressed in synovial tissue and peripheral blood monocytes from patients with rheumatoid arthritis (25,26). An in vitro study showed that pharmacologic up-regulation of HO-1 led to reduced production of inflammation markers in LPS-treated synovial cell lines (25). In contrast, suppression of endogenous HO-1 by anti–HO-1 small interfering RNA (siRNA) potentiated the proinflammatory effects of cytokines in isolated synovial cells. HO-1 is also expressed in normal and osteoarthritic human chondrocytes (27).

Taken together, the above results support the notion that HO-1 plays a pivotal role in modulating inflammation. However, the function of HO-1 is still debated. Devesa et al recently showed, in 2 different models of chronic autoimmune arthritis, that overexpression of HO-1 was ineffective in impeding the progression of the chronic inflammatory disease (28–30). The role of HO-1 in vivo in the acute phase of forms of arthritis that do not involve immunologic influence, however, has not been studied in depth. In addition, no previous studies, regardless of the animal model used, have included specific inhibition of HO-1 activity. To investigate whether HO-1 might have therapeutic potential in human inflammatory arthritis, we used a mouse model of nonautoimmune arthritis to determine, in vivo, the consequences of pharmacologic up-regulation of HO-1 and of its inhibition by injection of an anti–HO-1 siRNA.

**MATERIALS AND METHODS**

### Animals.
K/BxN mice were generated by crossing KRN-TCR–transgenic mice (B10.BR genetic background) with NOD mice (31). Expression of the αβββ-TCR transgene in (KRN × NOD) F1 offspring was identified by flow cytometry; these mice were found to correspond to arthritic K/BxN mice (32). Control K/BxN mice were arthritis-free F1 offspring that did not express the transgene. Two pools of sera were prepared, from arthritic and control K/BxN mice. BALB/c mice were injected intraperitoneally with arthritic or control K/BxN serum. Mice were kept under specific pathogen–free conditions in a clean room at the animal facility of the UFR Biomédicale des Saints Pères (Université Paris Descartes). Experimental protocols were approved by the Institut National de la Santé et de la Recherche Médicale Committee for Animal Studies.

### Induction and evaluation of arthritis.
Arthritis was induced in BALB/c mice by intraperitoneal injection of 100 μl serum from arthritic K/BxN mice on day 0 and day 1. Control mice were injected with 100 μl serum from control K/BxN mice. HO-1 was induced by intraperitoneal injection of CoPP (25 mg/kg body weight, in DMSO) in addition to serum transfer. Vehicle control animals received the same amount of DMSO alone. Paw thickness was measured daily, on days 0–7, using a dial gauge, and was assessed by investigators who were blinded with regard to treatment group.

### Determination of prostaglandin E2 (PGE2), IL-1β, IL-6, and TNFα concentrations in serum.
The PGE2 concentration in mouse serum was determined with the use of an enzyme immunoassay kit (EIA Kit-monoclonal; Cayman Chemical, Ann Arbor, MI), and IL-1β, IL-6, and TNFα concentrations with the use of enzyme-linked immunosorbent assay (ELISA) kits (Biotrak ELISA System; Amersham Biosciences, Orsay, France).

### Determination of serum antioxidant status and nitrite levels.
The total antioxidant status of serum was determined by evaluating inhibition of the oxidation of ABTS substrate, using a kit from Calbiochem (Meudon, France). Briefly, in the presence of strong antioxidant activity in serum, a low level of oxidized ABTS is detected. Nitrite levels were determined by...
indirect measurement of nitric oxide (NO) production by the Griess reaction, specifically adapted for serum.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) zymography. Mouse serum (2 μL/sample) was electrophoresed at 4°C in 8% SDS–polyacrylamide gels containing 0.1% gelatin, under nonreducing conditions. Proteins in the gel were denatured by incubation with 2.5% Triton X-100 for 1 hour at 20°C. Gelatinolytic activity was monitored as previously described (33).

Western blot analysis for HO-1. Mice were killed, and tissue extracts from the liver, spleen, lung, and knee joints were isolated with the use of a buffer containing 10 mM HEPES (pH 7.9), 10 mM KCl, 1.5 mM MgCl2, 0.5% Nonidet P40, 1 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride. Samples were centrifuged at 14,000 g for 20 minutes at 4°C. The supernatants were removed and centrifuged 2 more times at 14,000 g for 20 minutes at 4°C. Supernatants were separated by 10% SDS-PAGE and then subjected to immunoblotting. Equal transfer of proteins was confirmed by staining the nitrocellulose with ponceau red (0.2% [weight/volume] H2O diluted 99:1 in 10% trifluoroacetic acid). After blocking, blots were incubated with rabbit anti–HO-1 antibody (Stressgen, Victoria, British Columbia, Canada) and then with horse-radish peroxidase (HRP)–conjugated secondary antibodies. The protein bands in the blots were detected using an enhanced chemiluminescence kit (Pierce, Interchim, France).

HO-1 activity assay. HO-1 activity in joint and liver protein extracts was evaluated spectrophotometrically, using hemin (as substrate) and excess biliverdin reductase from mouse liver cytosol (34). To prepare mouse liver cytosol, samples of liver were first rinsed in ice-cold 1.15% KCl–20 mM Tris HCl buffer (pH 7.4). The homogenate was then centrifuged at 5,000 g for 20 minutes at 4°C and then at 105,000 g for 1 hour at 4°C. The supernatant, which contained microsomal biliverdin reductase, was incubated for 30 minutes in the dark at 37°C; in a reaction mixture containing hemin, NADPH, G6P, G6PDH, and joint or liver protein extracts. Bilirubin production was measured spectrophotometrically and expressed as picomoles of bilirubin per milligram of protein per hour.

Inhibition of HO-1 expression in vivo by siRNA. Small interfering RNA (siRNA) specifically targeted against HO-1 and nonspecific RNA were delivered using high pressure (35). Fifty micrograms of siRNA diluted in a final volume of 1 ml was delivered by rapid (10-second) injection into the tail vein on day 0, 1 hour before intraperitoneal injection of CoPP. Anti–HO-1 siRNA nucleotide sequences (36) were as follows: sense 5′-AAGCCACACAGCAGCAGCAGUGUAdTdT-3′, antisense 5′-UUACAUAGCUUGUGGCGGCUdTdT-3′. Nonsilencing siRNA was provided by Qiagen (Courtaboeuf, France). Sequences were as follows: sense 5′-UUCUCCGAAACGU-GUCACGUdTdT-3′, and antisense 5′-ACUGACACGUG-CGGAGAAdTdT-3′.

**Figure 1.** Induction of heme oxygenase 1 (HO-1) by cobalt protoporphyrin IX (CoPP) and its suppression by small interfering RNA (siRNA) in the joints, lung, and liver of BALB/c mice. A, BALB/c mice were injected intraperitoneally with CoPP (25 mg/kg) or DMSO vehicle. After 24 hours, mice were killed and HO-1 expression was assessed by Western blotting. B, HO-1 activity was assayed spectrophotometrically in 3 mice treated as described in A. C, Mice were injected intravenously in the caudal vein with nonspecific (ns) or anti–HO-1 siRNA 1 hour before CoPP injection. After 24 hours, mice were killed and HO-1 expression was assessed by Western blotting. D, HO-1 activity was assayed spectrophotometrically in 3 mice treated as described in C. Equal loading of protein for Western blotting was confirmed by ponceau red staining. Results shown in A and C are from 1 experiment representative of 3 experiments; results shown in B and D are the mean and SEM. **P < 0.01.
Histochemistry and immunohistochemistry. After the mice were killed, paws were dissected and fixed in 10% buffered formalin. Fixed tissues were decalcified, dehydrated, and embedded in paraffin. Sagittal sections (5 μm) were stained with hematoxylin and eosin. Some sections were incubated with rabbit inducible NO synthase (iNOS) antibodies (1:200 dilution; Transduction Laboratories, Lexington, KY) or Ki-67 antibodies (1:1,000 dilution; Novocastra, Newcastle, UK) and then with HRP-conjugated secondary antibodies. Ki-67 immunostaining is positive during the cellular proliferation process. Sections were counterstained with hematoxylin. For immunohistochemical controls, sections were incubated with phosphate buffered saline without the primary antibodies.

Determination of cartilage degradation by evaluation of the production of C-propeptide of type II collagen (PIICP). Cartilage degradation in mouse serum was assessed using a commercial ELISA for the PIICP epitope (Ibex, Montreal, Quebec, Canada). This assay measures the neoepitope created by cleavage of type II collagen following the release of newly synthesized procollagen into the matrix. Increased PIICP levels indicate increased cartilage degradation.

Statistical analysis. Each experiment was performed at least 3 times. The results are expressed as the mean ± SEM. In experiments comparing results obtained at different time points, statistical analysis was performed by analysis of variance and Tukey’s post hoc comparison. In all other experiments, the Wilcoxon signed rank test was used. P values less than 0.05 were considered significant.

RESULTS

Response of the HO-1 gene to CoPP induction and siRNA inhibition in BALB/c mice. BALB/c mice were injected intraperitoneally with CoPP or vehicle (DMSO). HO-1 levels in the joints and liver were analyzed by Western blotting 24 hours after injection, and the corresponding HO-1 enzymatic activity was assessed. HO-1 was detected in joint and liver extracts from treated mice (Figure 1A). The mean ± SEM enzyme activity of HO-1 in joints and liver extracts from 3 mice treated with CoPP was 349 ± 59 and 354 ± 75 pmoles/mg/hour, respectively (Figure 1B). Vehicle alone had no effect on HO-1 expression or activity.

In another set of experiments, anti–HO-1 siRNA or nonspecific siRNA was injected intravenously into the caudal vein of mice 1 hour before CoPP injection. Compared with nonspecific siRNA, the anti–HO-1 siRNA elicited a decrease in HO-1 levels in the joints, lungs, and liver, but not the spleen (Figure 1C). Similarly, the anti–HO-1 siRNA elicited a reduction in HO-1 activity levels in joint, lung, and liver extracts (102 ± 43 pmoles/mg/hour, 210 ± 60 pmoles/mg/hour, and 166 ± 48 pmoles/mg/hour, respectively), but not in the spleen (Figure 1D). In further experiments, CoPP and anti–HO-1 siRNA were used to modulate HO-1 expression in mice with K/BxN serum–induced arthritis.

Suppression of clinical manifestations of joint inflammation in K/BxN serum–induced arthritis by HO-1. BALB/c mice were injected with serum from transgenic K/BxN mice exhibiting arthritis symptoms.
(K/BxN serum) or with control serum from asymptomatic K/BxN mice, along with CoPP or the corresponding DMSO vehicle. Local inflammation of the joints was quantified by measuring paw thickness daily for 7 days (Figure 2A). In mice injected with K/BxN serum plus vehicle, paw thickness was significantly increased on day 3 (mean ± SEM 4.6 ± 0.2 mm, versus 3.9 ± 0.1 mm in mice treated with control serum plus vehicle; P < 0.05). Joint inflammation progressed continuously from day 4 to day 7 in K/BxN plus vehicle–injected mice (paw thickness 4.7 ± 0.2 mm on day 7; P < 0.01 versus mice treated with control serum plus vehicle), but not in mice treated with K/BxN serum plus CoPP (4.1 ± 0.1 mm on day 7) or in control mice (3.9 ± 0.1 mm on day 7).

The groups administered control serum plus vehicle or K/BxN serum plus CoPP exhibited no symptoms throughout the 7-day period, and no significant differences in paw thickness between these 2 groups were observed on day 7. Sham-treated mice and mice treated with control serum and CoPP showed no significant difference in paw thickness compared with those treated with control serum and vehicle (data not shown).

Since CoPP is not a specific inducer of HO-1, we specifically inhibited HO-1 in vivo in CoPP- and K/BxN-treated mice by siRNA treatment (Figure 2B). Mice were injected intravenously with an anti–HO-1 siRNA or a nonspecific siRNA sequence as a control, 1 hour before injection of K/BxN serum and CoPP. Paw thickness was assessed as described above. As shown in Figure 2B, the paws of mice treated with anti–HO-1 siRNA were thicker than those of mice receiving nonspecific siRNA (P < 0.01). Between day 0 and day 7, paw thickness was not affected in the mice treated with the nonspecific siRNA, which suggests that siRNA treatment does not interfere with paw thickness. These results strongly indicate that the protective effect of CoPP on acute joint inflammation is specifically mediated by HO-1.
HO-1–induced attenuation of the systemic inflammation elicited by K/BxN serum. In BALB/c mice injected with K/BxN serum or control serum in conjunction with CoPP or vehicle, serum PGE\textsubscript{2} content and gelatinolytic activity were measured as indices of systemic inflammation, at the onset of the inflammatory process (days 0–3) and 7 days after arthritis induction. On days 1, 2, and 3 after injection with K/BxN serum, the PGE\textsubscript{2} concentration was increased (mean ± SEM 111 ± 10 ng/ml, 80 ± 4 ng/ml, and 71 ± 6 ng/ml, respectively), as compared with levels in mice injected with control serum (Figure 3A). Concomitant injection with CoPP significantly reduced the level of PGE\textsubscript{2} on days 1, 2, and 3, to 72 ± 5 ng/ml, 60 ± 6 ng/ml, and 52 ± 3 ng/ml, respectively ($P < 0.01$ versus levels in mice concomitantly injected with vehicle). In parallel, CoPP cotreatment reduced 92-kd gelatinolytic activity (matrix metalloproteinase 9 [MMP-9]), whereas MMP-2 activity remained constant (Figure 3B). The antiinflammatory effects of CoPP on serum PGE\textsubscript{2} levels and MMP-9 activity were also observed on day 7.

We then specifically inhibited HO-1 in vivo in CoPP and K/BxN serum–treated mice, by siRNA treatment as described above. PGE\textsubscript{2} production (Figure 3C) and MMP-9 activity (Figure 3D) were significantly higher in the presence of the anti–HO-1 siRNA as compared with the nonspecific siRNA. Again, these data provide strong evidence that the protective effect of CoPP on systemic inflammation is essentially mediated by HO-1.

HO-1 attenuates systemic cytokine production and increases systemic antioxidant status but has no effect on systemic NO production elicited by K/BxN serum. A similar protocol involving CoPP and siRNA treatment of K/BxN serum–treated mice was carried out to investigate serum levels of the major inflammatory cytokines involved in the acute phase of nonautoimmune arthritis. As shown in Figure 4A, K/BxN serum treatment induced production of IL-1$\beta$, TNF\textalpha, and IL-6 (mean ± SEM 45 ± 2 pg/ml, 23 ± 3 pg/ml, and 1,767 ± 300 pg/ml, respectively), which was reduced to basal levels by CoPP cotreatment. The antiinflammatory effects of CoPP were suppressed by treatment with anti–HO-1 siRNA injection (Figure 4B). The nonspecific siRNA had no effect. A similar phenomenon was observed with regard to the antioxidant status of serum. As shown in Figure 4A, cotreatment with CoPP and K/BxN increased the antioxidant activity by a factor of 2.7 as compared with treatment with K/BxN alone. This systemic effect of CoPP on antioxidant status was suppressed by injection of anti–HO-1 siRNA (Figure 4B). No similar effect on systemic NO production was observed (Figures 4A and B).

These findings demonstrate that the protective effect of HO-1 is mediated mainly by significant reduction of proinflammatory cytokine production and up-regulation of systemic antioxidant activity, but not by regulation of systemic NO production. In addition, these data, along with results presented above, indicate that the protective effects of CoPP occur in the early phase of
induced arthritis and are sustained for at least 7 days and mediated mainly by HO-1.

**HO-1–induced reduction of local tissue inflammation and cellular recruitment elicited by K/BxN serum.** Examination of histologic specimens from BALB/c mice injected with K/BxN serum or control serum in conjunction with CoPP or vehicle revealed the presence of inflamed joint tissue in animals treated with K/BxN serum, but a total absence of inflammation in the joints of K/BxN serum–treated animals that received concomitant CoPP treatment or control mice (Figure 5). In parallel, we observed iNOS staining only in inflamed joint tissue from mice treated with K/BxN serum alone (Figure 5). No staining for Ki-67, a usual marker of cell proliferation, was observed under any of the experimental conditions (results not shown), indicating that there was no modulation of cell proliferation and that the effect of HO-1 more likely involves cell recruitment. These findings suggest that HO-1 could protect against joint tissue inflammation at the local level by down-regulating iNOS and reducing cell recruitment.

**HO-1 attenuates cartilage degradation but does not inhibit chondrocyte iNOS expression elicited by K/BxN serum.** Cartilage degradation was assessed by measuring serum levels of PIICP on day 7. Circulating levels of the PIICP epitope were reduced in CoPP-cotreated mice (mean ± SEM 46 ± 6 ng/ml) as compared with mice treated with K/BxN serum alone (68 ± 8 ng/ml) (Figure 6A), showing that CoPP attenuates cartilage degradation induced by K/BxN serum. Finally, positive immunostaining of articular chondrocytes for iNOS was observed in mice treated with K/BxN serum alone and also, surprisingly, in mice treated with K/BxN serum plus CoPP (Figure 6B). These results and those described above indicate that, although it is locally protective against joint tissue inflammation and cartilage degradation, HO-1 is still unable to inhibit iNOS expression in articular chondrocytes.

**DISCUSSION**

The purpose of the present work was to analyze whether in vivo induction of HO-1 during the acute
phase of inflammatory arthritis in mice leads to protective antiinflammatory effects, including attenuation of systemic proinflammatory cytokine induction and joint destruction. We used the K/BxN mouse model of induced arthritis, which mimics human inflammatory arthritis without any lymphocyte influence. HO-1 induction by CoPP suppressed the development of arthritis induced by K/BxN serum in BALB/c mice. CoPP also attenuated joint destruction, PGE2 production, and MMP-9 activity, as well as serum IL-1β, IL-6, and TNFα production. These effects were largely abolished in vivo in the presence of an anti–HO-1 siRNA.

The original K/BxN mouse model was a model of spontaneous arthritis, described in 1996 by Kouskoff et al (37). In 1999, an alternative model based on serum transfer from arthritic K/BxN mice into healthy animals was introduced by the same group (31). As pointed out in a recent report by Ohmura et al (38), this latter model provides a powerful experimental tool for analyzing the acute effector-phase pathways in arthritis, without the confounding influence of the immunologic phase. This serum transfer model is simple, robust, produces 100% arthritis incidence in BALB/c mice, and provides a rapid (24-hour) response (31). We used the K/BxN mouse serum transfer model because the primary intent of the present study was to investigate in a broad sense the innate acute inflammatory arthritic process observed in human inflammatory arthritis, without lymphocyte influence.

The poor specificity of HO-1 modulators (CoPP, hemin, zinc protoporphyrin IX, and tin protoporphyrin IX) used either in vivo or in vitro, as well as the lack of efficient breeding of HO-1–null mice, are sources of experimental discrepancies. Metalloporphyrin inhibitors of HO-1 robustly affect enzymes such as NOS and guanylate cyclase, in addition to their various other nonspecific effects (39,40). Thus, to evaluate the exact role of HO-1 in the effects of CoPP observed in the K/BxN mouse model of arthritis, we developed an anti–HO-1 siRNA and a method for its systemic deliv-
The finding that iNOS was still expressed in chondrocytes but the effect on cartilage was only partial, as evidenced by regulation reduced inflammatory joint tissue proliferation and subchondral bone tissue. In the present study, HO-1 up-regulation may not be beneficial in chronic autoimmune arthritis but may have real therapeutic potential during the acute phase of arthritis while we chose the K/BxN serum transfer model, which mimics the acute, non-autoimmune phase of inflammatory arthritis.

Besides the lymphocyte influence, another explanation for the discrepancies observed with the autoimmune and nonautoimmune arthritis models may relate to qualitative and quantitative differences in the HO-1 inducers used. Devesa et al used CoPP at 2.5 mg/kg body weight in the collagen-induced arthritis model and 5 mg/kg in the rat adjuvant-induced arthritis model (28,29), whereas we used 25 mg/kg in our model. Using hemin, another HO-1 inducer, Devesa et al observed no response in rat adjuvant-induced arthritis (28), a result which differed from findings reported by Kobayashi et al (25). Conversely, our results on HO-1-induced protective effects and their suppression by a specific siRNA parallel the in vitro findings of Kobayashi and colleagues in studies performed on an LPS-treated fibroblast-like synoviocyte cell line in culture (25). Overall, our findings, in accordance with the existing literature, suggest that strategies aimed at HO-1 expression in the process. Our authors showed that pharmacologic up-regulation of HO-1 by CoPP was not sufficient to control the progression of chronic inflammation in rat adjuvant-induced arthritis and mouse collagen-induced arthritis (28,29), whereas inhibition of the endogenous form of HO-1 significantly reduced the severity of arthritis and the production of inflammation mediators in rat adjuvant-induced arthritis (30). These discrepancies appear to us to be essentially related to the different models of arthritis. Devesa and colleagues used classic models specifically designed to study the late immunologic phase of arthritis while we chose the K/BxN serum transfer model, which mimics the acute, non-autoimmune phase of inflammatory arthritis.

In conclusion, this study is the first to demonstrate that the acute joint inflammatory response induced by K/BxN serum in BALB/c mice can be counteracted by pharmacologic up-regulation of HO-1 by CoPP. This chemical inducer elicits a robust decrease in joint destruction. This decrease is abolished in the presence of anti–HO-1 siRNA, which demonstrates the essential involvement of HO-1 expression in the process. Our results strongly support the notion that HO-1 has a potential role in treatment during the acute phase of human inflammatory types of arthritis, such as acute gout and pseudogout, infectious arthritis, seronegative spondylarthritides, rheumatoid arthritis, and juvenile arthritis.

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AUTHOR CONTRIBUTIONS

Dr. Rannou had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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