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Anti-interleukin-1 effects of diacerein and rhein in human osteoarthritic synovial tissue and cartilage cultures

By Michael Yaron, Idit Shirazi and Ilana Yaron
Arthritis Research Unit, Department of Rheumatology, Tel Aviv-Sourasky Medical Center, Ichilov Hospital, and Tel Aviv University Sackler Faculty of Medicine

Summary

Objective: The etiology of osteoarthritis (OA) is still a matter of debate. Several factors are known to be involved in the destruction of the articular cartilage. Interleukin-1 (IL-1) plays an important role in the pathogenesis of osteoarthritis (OA) either directly or through the stimulation of catabolic factors, such as nitric oxide (NO). The objective of this study was to evaluate the effect of diacerein, a new anti-OA agent and its active metabolite, rhein, on the production and function of IL-1β, nitric oxide (NO) and receptor agonist (IL-1ra) in human OA cartilage and synovial tissue cultures.

Design: Synovial tissue and cartilage derived from OA patients were kept in culture for 48–72 hours in the presence of 1 μg/ml of lipopolysaccharide (LPS) with or without diacerein (10^{-7}–10^{-5} M), rhein (10^{-7}–10^{-5} M) and hydrocortisone (5 μg/ml). IL-1β, IL-1ra, NO productions and ^{35}S uptake were measured in culture media. In some experiments the resulting supernatants from synovial tissue cultures were added to cartilage.

Results: Diacerein and rhein, as well as hydrocortisone, significantly inhibited LPS-induced IL-1β production by synovial tissue and cartilage. They also significantly reversed the inhibitory effect of LPS on cartilage ^{35}S uptake. Culture media from synovial tissue containing LPS+diacerein (10^{-6} M) or +rhein (10^{-6} M) had a significantly less inhibitory effect on cartilage synthesis than culture media containing LPS only. Diacerein and rhein decreased NO release in synovial tissue and cartilage media and increased IL-1ra levels in cartilage culture media.

Conclusion: An inhibitory effect of diacerein and rhein at therapeutic concentrations on both IL-1β secretion and function in human synovial tissue and cartilage is suggested. Diacerein and rhein effects on NO production by LPS-stimulated OA synovial tissue and cartilage may both contribute and elucidate their anti-OA properties.

Key words: Diacerein, Rhein, Anti-IL-1, Human OA synovial tissue and cartilage.

Introduction

Osteoarthritis (OA) is a slowly progressive disease of unknown cause and obscure pathogenesis. It is clinically characterized by pain, deformity, enlargement of the joints, loss of joint stability and limitation of motion. Pathologically, the disease is characterized by focal destructive cartilage lesions, subchondral sclerosis, cyst formation and large osteophytes at the margins of the joint. The disease appears to originate in the cartilage and changes in this tissue are progressively severe with advancing age [1]. Therapeutically, the disorder is characterized by the lack of a specific healing agent.

Although the etiology of OA is still a matter of debate, several factors are known to initiate and/or contribute to the breakdown of the articular cartilage. These include the production of cytokines [2] and superoxide oxygen free radicals [3], the release of proteolytic enzymes [4, 5], the impairment of collagen and proteoglycan synthesis [6, 7], deposition of immuno-complexes and microcrystals [8, 9] and synovial inflammation [10, 11].

In OA, both mechanical and enzymatic factors are involved in cartilage matrix degradation. The enzymatic process appears to be related to a cascade of events with the chondrocyte being the most important source of cartilage degradation enzymes. The most important enzymes appear to be metalloproteases. Collagenase and stromelysin are the two major metalloproteases involved in cartilage breakdown. Their levels are increased in OA synovial fluid [12].

The regulation of chondrocytes secretory function and their proliferation by cytokines and growth factors is therefore central to cartilage development and the maintenance of homeostasis in the joint [13].

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Address correspondence and reprint requests to: Michael Yaron, Professor and Head, Department of Rheumatology, Ichilov Hospital, 6 Weizmann Street, Tel Aviv 64239, Israel.

The action of interleukin-1 (IL-1) on articular cartilage is multifaceted and it most likely plays an important role in the mechanism of cartilage destruction. IL-1 suppresses the synthesis of the cartilage matrix components, mainly collagen type II [14] and aggregating proteoglycan (aggrecan) [15, 16]. The ability of IL-1 to promote the degradation of cartilage matrix macromolecules is linked to its ability to promote the synthesis and secretion of matrix proteases, including tissue plasminogen activator, collagenase and stromelysin by chondrocytes [17]. The production of prostaglandin E_2 (PGE_2) by chondrocytes is also stimulated by IL-1 [18, 19]. Overproduction of PGE_2 plays a role in exacerbating joint inflammation, stimulating bone resorption and modulating the immune response. IL-1 inhibits chondrocyte hypertrophy and the onset of calcification in ossifying cartilage [20] and chondrocyte proliferation induced by serum or transforming growth factor β (TGF β) [21].

In addition to cartilage, IL-1 affects the function of other articular tissues. Synovial cells also exhibit stimulation of metalloproteases and PGE_2 production in response to IL-1 [22]. IL-1 is considered a major factor of chondrocyte and synoviocyte activation since it induces the synthesis of neutral metalloproteases and promotes the destruction of macromolecules in the matrix [23] and is amply involved in cartilage degradation and in synovial inflammation.

Other factors implicated in the degradative process of cartilage are also linked to cytokine activity. Nitric oxide (NO) production is part of the catabolic process in cartilage metabolism and may therefore play a role in OA pathogenesis [24, 25].

Drugs which interfere with factors known to initiate and/or contribute to the breakdown of the articular cartilage may provide therapeutic benefit in the treatment of OA. Diacerein, a novel drug for the treatment of osteoarthritis, showed anti-OA effects in experimental animal OA models [26–28]. In humans, diacerein showed beneficial clinical effects on the symptoms of OA patients [29, 30]. Diacerein is marketed in France by Negma as ART 50®. Diacerein is currently under evaluation as a disease-modifying OA drug (DMOAD) in a 3-year, randomized, double-blind, placebo-control clinical study of 500 patients with OA of the hip (ECHODIAH study) [31].

Diacerein has no effect on phospholipase A_2, cyclo-oxygenase and 5-lipoxygenase but antagonizes IL-1 effects on cartilage matrix components and collagenolytic activity [32–35]. In other studies diacerein and its active metabolite, rhein, have been shown to inhibit superoxide release from human neutrophils [36] and to reduce the migration and phagocytic activity of mouse peritoneal macrophages [37].

The present study was designed to evaluate in-vitro effects of diacerein and rhein on IL-1β, NO and interleukin-1 receptor agonist (IL-1ra) production in human osteoarthritic synovial and articular cartilage cultures in order to further elucidate their mechanism of action in OA.

Materials and methods

CHEMICALS AND BIOCHEMICALS

Diacerein and rhein were dissolved in absolute ethanol and DMSO, respectively. The final ethanol or DMSO concentrations in experimental wells did not exceed 0.04%. Lipopolysaccharide (LPS) at a final concentration of 1 μg/ml (Escherichia coli serotype 0127: B8) was from Difco Laboratories (Detroit, MI, U.S.A.), hydrocortisone as a water soluble sodium succinate ester (Solu Cortef™ sterile powder) was from Upjohn s.a (Belgium), indomethacin from Sigma (St Louis, MO, U.S.A.), recombinant human interleukin-1β from Biogen SA (Geneva, Switzerland). DMEM and RPMI culture media were obtained from Biological Industries (Beit Haemek, Israel).

SPECIMEN SELECTION AND CULTURE CONDITIONS

Synovial tissue and cartilage were obtained in the operation theater from patients undergoing total knee or hip replacement for osteoarthritis (11 males, age 66.8 ± 6.7 years; 17 females, age 70.4 ± 1.4 years, mean ± SD).

Organ cultures

Within 2 hours of removal, synovial tissues were cut into small pieces, a few millimeters in diameter and approx. 80 mg synovial tissue pieces were cultured in 2 ml RPMI 1640 supplemented with L-glutamine (2 mM), penicillin (100 U/ml) and streptomycin sulphate (100 μg/ml) (Biological Industries) with test materials. Following 48 hours of incubation (37°C, 5% CO_2), the measurement of IL-1β, nitric oxide (NO) and IL-1ra production was performed in supernates.

Cartilage tissue (without distinction between 'normal' and diseased areas) was finely diced, randomized and distributed in 96-well plates containing approx. 15 mg cartilage pieces per well in 0.2 ml DMEM, 1% FCS, penicillin and streptomycin. Test materials were added and followed 24

hours later by carrier free $^{35}SO_4$ (Amersham, Buckinghamshire, U.K., specific activity 1100 Ci/mmol) at a final concentration of 40 μCi/ml. After two days of further incubation the supernates and cartilage were collected for ^{35}S-glycosaminoglycan (^{35}S-GAG) determination. IL-1β, IL-1ra and NO assays were performed in separate cultures with no radioactive material added (after 48 hours incubation).

^{35}S-GAG determination

Labeled ^{35}S-GAG was determined in proteolytic digests of combined cartilage tissue and culture medium by the cetyl pyridinium chloride fixation wash procedure [38, 39]. Digestion was performed at 55°C for 16 hours with pronase (0.5 mg/ml) in 0.05 M Tris buffer pH 7.6 (Sigma, St Louis, MO) in a total volume of 300 μl.

Experiments on cartilage were performed either with test materials added directly to cartilage culture or, as described in the legend of Fig. 4, drugs were added to synovial tissue cultures, incubated for two days and the resulting supernatants added to cartilage cultures. Untreated synovial tissue media (conditioned media) obtained in the same way were used as controls.

Interleukin-1β, interleukin-1 receptor antagonist and nitric oxide production determination

IL-1β and IL-1ra secreted into culture media were determined by quantikine human immunoassay technique (R&D Systems, Inc., U.S.A.), which uses the quantitative sandwich enzyme immunoassay technique. Detection limits were 1 pg/ml and 14 pg/ml, respectively.

NO was determined as previously described by Ashab *et al.* [40]: NO_2 and NO_3 were determined after the reduction of NO_3 to NO_2 by a 90 min incubation in a tilting bath (37°C) using nitrate reductase from *E. coli* and beta nicotinamide adenine dinucleotide phosphate (reduced form) (Sigma) as cofactor. NO_2 was determined with Griess reagent. Sensitivity of procedure was 3 μM.

Cell viability determination

Cell viability in the presence of diacerein and rhein (10^{-5}–10^{-7} M) was performed on human synovial fibroblast monolayer cultures after drug exposure for 72 h by trypan blue exclusion test.

Toxicity was evaluated by tritiated thymidine incorporation into DNA.

Calculation and statistical analysis

In synovial tissue and articular cartilage cultures, results of IL-1β, IL-1ra and NO determinations were expressed per mg tissue using tissue weights obtained at the end of experiments.

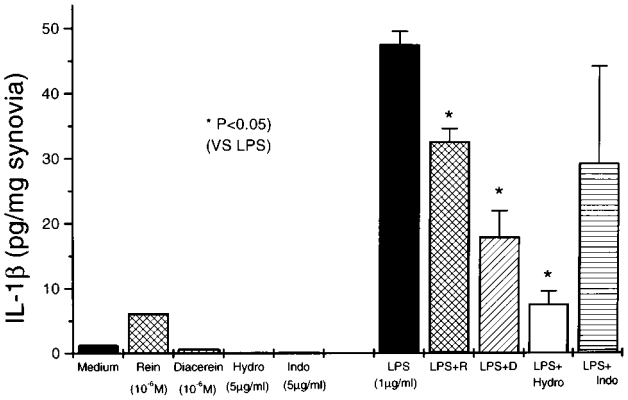

FIG. 1. Effect of diacerein (D) (10^{-6} M), rhein (R) (10^{-6} M), hydrocortisone (hydro) (5 μg/ml) and indomethacin (indo) (5 μg/ml) on IL-1β secreted by LPS stimulated human synovial tissue in culture. *P*-values (versus LPS) were as follows: **P*<0.05, ***P*<0.02, ****P*<0.001.

In general, results were verified in at least three experiments, each performed in triplicate or quadruplicate. In most cases data from a representative experiment are shown. However, where there was some variability between experiments, results from several experiments were pooled and expressed as percentage (Figs 6, 7). Statistical significance (*P*-determination) was evaluated by ANOVA and by a Student's t-test. A *P*-value less than 0.05 was considered statistically significant.

Results

In a typical experiment (Fig. 1), the effects of diacerein (10^{-6} M), rhein (10^{-6} M), hydrocortisone (10^{-5} M) and indomethacin (10^{-5} M) on IL-1β secretion were investigated on human OA synovial tissue in culture with and without LPS (1 μg/ml).

In the absence of LPS, IL-1β levels in control culture media were low (~1 pg/ml); test materials without LPS did not show significant changes compared to controls (Fig. 1). LPS added to cultures induced a 47-fold increase of IL-1β production by human OA synovial tissue. Both diacerein and rhein induced a significant inhibition of IL-1β production stimulated by LPS. Hydrocortisone and indomethacin also had an inhibitory effect in this respect, and only reached a statistical significance for hydrocortisone (Fig. 1).

In cultured human cartilage (Fig. 2), the effects of diacerein and rhein (10^{-7} to 10^{-5} M) on IL-1β secretion was assessed in the presence or the absence of LPS. Without LPS, IL-1β was poorly produced by cartilage and was amply secreted when LPS (1 μg/ml) was added to cultures (Fig. 2).

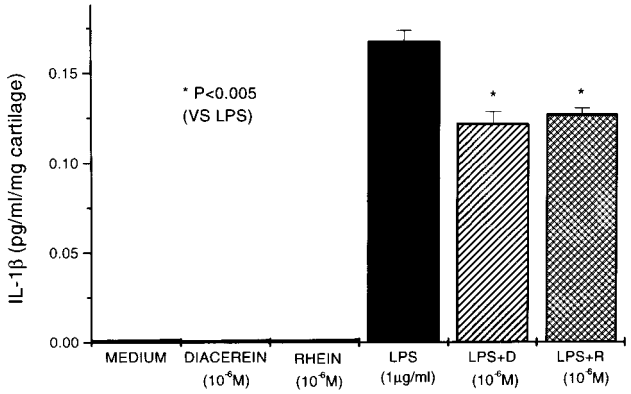

FIG. 2. Effect of diacerein (D) and rhein (R) (10^{-7}–10^{-5} M) on IL-1β secreted by LPS stimulated human cartilage in culture. Significance was versus LPS: *P<0.05.

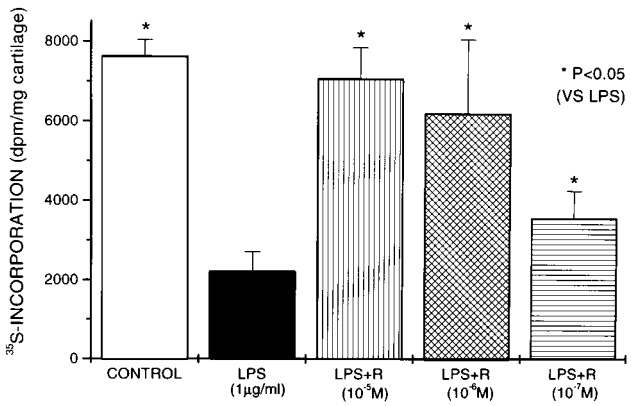

FIG. 3. Effect of rhein (R) (10^{-7}–10^{-5} M) on LPS inhibition of human cartilage ^{35}S-GAG synthesis. Significance was versus LPS: *P<0.05, ***P<0.0001.

In these conditions, diacerein and rhein induced a dose-dependent decrease of IL-1β production stimulated by LPS in cultured human cartilage with statistical significance at 10^{-6} and 10^{-5} M.

The effect of three concentrations of rhein on proteoglycan synthesis was studied in human cartilage in culture (Fig. 3). LPS (1 μg/ml) induced a remarkable decrease of ^{35}S incorporation by cartilage. Rhein significantly reversed, in a dose-dependent manner, this inhibition and counteracted the inhibitory effect of LPS on the proteoglycan synthesis. Diacerein at equivalent concentrations was 89 ± 24% as effective as rhein in reversing inhibition (data not shown).

The influence of conditioned media from human OA synovial tissue, kept in culture, on ^{35}S incorporation by cultured cartilage was assessed (Fig. 4). Conditioned media from synovial tissue cultured in the presence of LPS for 72 hours inhibited ^{35}S incorporation into human articular cartilage (N=4) (Fig. 4). The presence of diacerein (10^{-6} M), rhein (10^{-6} M) or hydrocortisone

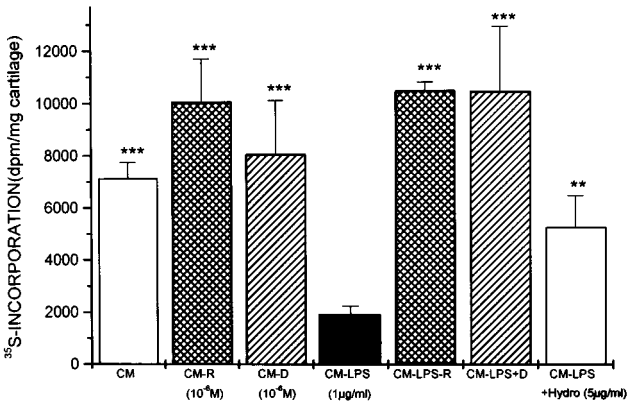

FIG. 4. Effects of synovial tissue media on ^{35}S uptake by human cartilage cultures. Drugs were added to synovial cell tissue cultures, incubated for two days and the resulting supernatants (conditioned media=CM) added to cartilage cultures either alone or with LPS and no test materials (CM-LPS), with LPS and diacerein (C − LPS+D), with LPS and rhein (CM − LPS+R), with LPS and hydrocortisone (CM–LPS+hydro). P-values (versus LPS) were: **P<0.02, ***P<0.001.

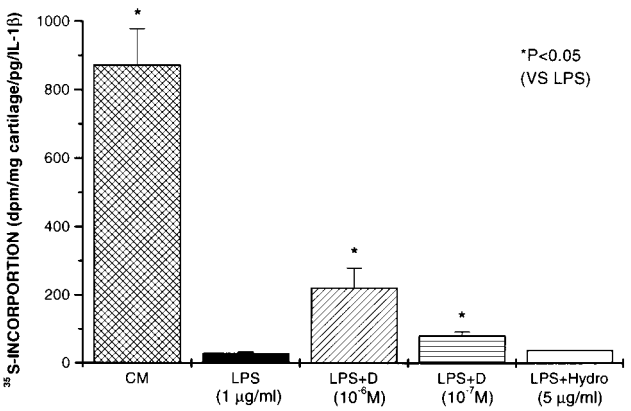

FIG. 5. Effect of diacerein (D) (10^{-5} M) and rhein (R) (10^{-6} and 10^{-5} M) on nitric oxide (NO) release into the media of LPS stimulated human synovial tissue in culture. Significance versus LPS was: *P<0.05, **P<0.002. NO represents combined measurements of NO_3 and NO_2 as described in Materials and Methods.

(10^{-5} M) with LPS in human OA synovial media significantly reversed the inhibitory effect of conditioned media with LPS alone on ^{35}S uptake by human cartilage cultures (Fig. 4).

The effects of diacerein and rhein on NO concentration in culture media of human synovial tissue and human articular cartilage are presented in Figs 5, 6. LPS (1 μg/ml) added to the culture media induced a 27% and 200% increase of the NO production in synovial tissue and cartilage cultures, respectively. Diacerein and rhein decreased LPS stimulated NO concentrations in both cultures. Inhibition was statistically significant at 10^{-5} M drug concentration.

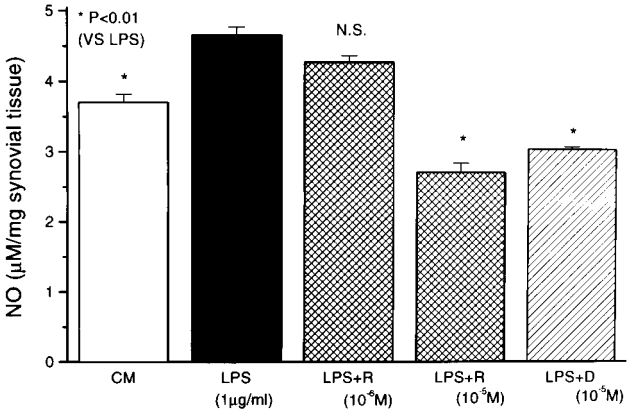

FIG. 6. Effect of diacerein (D) and rhein (R) (10^{-6} and 10^{-5} M) on NO levels in media of LPS stimulated human articular cartilage cultures. Results are expressed versus a 100% LPS stimulated human cartilage culture for four independent experiments. Mean absolute values were for control 6.3 μM/mg cartilage and for LPS 19.8 μM/mg cartilage. Significance versus LPS was: $*P<0.05$, $***P<0.001$.

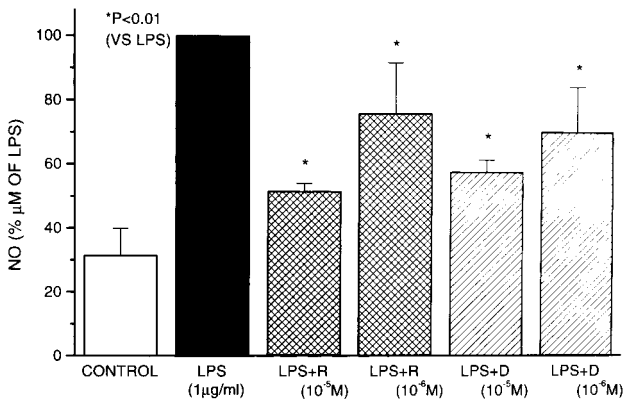

FIG. 7. Effect of diacerein (D) (10^{-6} and 10^{-5} M) and rhein (R) (10^{-6} M) on interleukin-1 receptor antagonist (IL-1ra) in synovial tissue cultures. Results are expressed versus a 100% LPS stimulated human synovial tissue culture for four independent experiments. Mean absolute values were for control 117 pg/mg synovia and for LPS 434 pg/mg synovia. Significance versus LPS was: $**P<0.02$, $***P<0.001$.

The effect of diacerein and rhein on IL-1ra were studied in synovial tissue and cartilage cultures (Figs 7, 8). In both cultures, LPS (1 μg/ml) stimulated the IL-1ra concentration in the medium. Diacerein and rhein significantly inhibited IL-1ra released in synovial culture media (Fig. 7), but stimulated its concentration in media of human cartilage cultures (Fig. 8).

Diacerein and rhein, added to synovial fibroblast monolayer cultures at concentrations of 10^{-6} and 10^{-5} M did not show any deleterious effect on cell viability which remained >90% in comparison with controls.

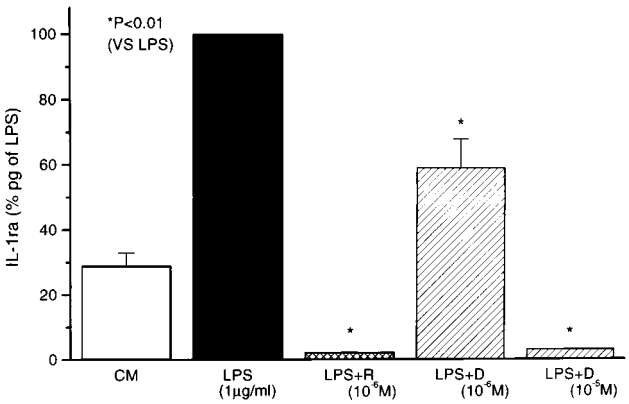

FIG. 8. Effect of diacerein (D) and rhein (R) (10^{-6} and 10^{-5} M) on interleukin-1 receptor antagonist (IL-1ra) secreted into the media of LPS stimulated human articular cartilage. Significance versus LPS was: $*P<0.05$, $**P<0.002$, $***P<0.001$.

Discussion

The effects of diacerein and rhein on IL-1β, IL-1ra and NO production in human OA synovial and cartilage cultures and their action on cartilage proteoglycan synthesis in the presence of an IL-1 inducer (LPS) are reported in this study. Experiments were performed in an in-vitro model using in some of them a combination of human synovial tissue media and human articular cartilage cultures in order to mimic in part the in-vivo situation. The objective of this study was to clarify the mechanism of action of diacerein and rhein on human OA cartilage.

IL-1 is one of the important factors involved in cartilage destruction in osteoarthritis. The presence of both IL-1α and IL-1β in OA synovium and cartilage has been documented and previously reported using immunohistochemical techniques [41, 42]. Since IL-1 can induce resorption of cartilage [43], it is tempting to presume that its involvement in the etiology of OA very likely, but the precise cascade of events resulting in cartilage damage is yet to be clarified. However, much evidence suggests that IL-1 can modulate chondrocyte metabolism, including synthesis of matrix macromolecules and proteases.

In our model, diacerein and rhein did not exhibit any effect on IL-1β production in the absence of LPS. LPS is considered to promote inflammation [44], probably by stimulating production of cytokines such as IL-1 and TNFα [45]. When synovium and cartilage were treated with LPS, IL-1β production was induced. Diacerein and rhein significantly inhibited this stimulation of IL-1β production at therapeutic doses. This confirms the findings of Pelletier *et al* indicating that diacerein and its metabolite, rhein, inhibited the synthesis of

IL-1β [46]. Diacerein and rhein counteracted the inhibitory effect of LPS on cartilage ^{35}S-GAG synthesis (Figs 3, 4), which may be related to their inhibition of IL-1 production.

Both drugs, as well as hydrocortisone, lowered IL-1β levels in LPS-stimulated synovial conditioned media and reversed the inhibitory effect of synovial conditioned media on cartilage matrix synthesis.

IL-ra is a protein produced by several cell types, including monocytes, synoviocytes and chondrocytes, and is able to block several of the effects of IL-1 [47]. In our experiments, LPS stimulated the production of IL-1ra in synovial tissue and cartilage cultures, as reported in a previous study [48]. Its stimulated secretion in the media of human cartilage cultures induced by diacerein and rhein may explain at least in part the beneficial effects of these drugs on cartilage matrix constituents in OA. Our results are consistent with those of Caron *et al.* who demonstrated that intra-articular injections of recombinant human IL-1 receptor antagonist can protect against the development of OA lesions by a reduction of collagenase expression [49]. An intriguing finding described in Figs 7 and 8 is that rhein and diacerein significantly inhibit LPS induced IL-1ra production in OA synovial tissue cultures, while they significantly stimulate it in OA articular cartilage cultures. In addition, the amount of IL-1ra produced by 1 mg synovial tissue (117 pg/mg synovia and LPS stimulated 434 pg/mg synovia, see legend to Fig. 7) is much higher than that produced by 1 mg cartilage in culture (1 and 3 pg/mg cartilage, respectively, see Fig. 8). However, in early osteoarthritis the total amount of cartilage is much larger than that of the synovial membrane. This could suggest that at least from the point of view of their effect on IL-1ra production, rhein and diacerein would be more effective in early OA when the total mass of cartilage is much larger than that of synovial tissue. Furthermore, the *local* increased production of IL-1ra induced by diacerein and rhein in cartilage may be more important than the altered production of IL-1ra occurring in synovial tissue. In the same vein, results in Fig. 4 point out that conditioned media of synovial tissue in the presence of LPS and diacerein reverse inhibition of cartilage synthesis induced by LPS alone, in spite of stimulated IL-1ra in synovial tissue conditioned media.

In OA, the primary lesion affects the cartilage, while synovial inflammation is secondary and likely related to the action of several factors, including the release of cartilage matrix breakdown products and the presence of microcrystals in the synovial fluid and in the synovium [1, 50]. Diacerein and rhein induced an inhibition of both IL-1β and IL-1ra in the synovial tissue culture, while in cartilage culture, these agents inhibited IL-1β, counteracted the inhibitory effects of this cytokine on the ^{35}S-GAG synthesis and stimulated the production of IL-1ra. Considering these findings, the net effect of rhein and diacerein on the cartilage, which is the primary location of OA lesions, is beneficial. This is confirmed in different animal models of OA, where diacerein administered to animals exhibited chondroprotective activities [26–28].

Diacerein can have effects at the level of gene expression [51], translation and/or post-translation [46]. In a recent experiment, diacerein and rhein effects on IL-1β were found to be post-transcriptional by the absence of an effect on gene expression [46]. In the same study, the authors demonstrated that both drugs produced a decrease in the number of IL-1 receptors on OA chondrocytes: this is of importance because an increase in the number of IL-1 receptors per cell was reported in OA chondrocytes and synoviocytes, thus rendering human OA cartilage matrix synthesis sensitive to inhibition by IL-1 [52]. These findings together with the ability of diacerein and rhein to stimulate IL-1ra in cartilage culture may explain why diacerein also has an inhibitory action on IL-1 function in cartilage.

Increased levels of NO have been found in OA and rheumatoid arthritis human synovial fluid [53]. Synovial cells and chondrocytes are important sources of NO production [54, 55]. Its synthesis by chondrocytes and synoviocytes is stimulated by inflammatory cytokines such as IL-1 and TNF-α [56] and also by LPS [24]. In our study, both diacerein and rhein inhibited the production of NO after LPS stimulation of synovial tissue and cartilage cultures. Although its production is stimulated by cytokines, it might play a role, itself, by mediating IL-1 proteoglycan synthesis suppression in articular cartilage [16] and by reducing the synthesis of IL-1ra [57]. These observations stress the important role of NO as a mediator of the pathophysiological changes taking place in OA. Inhibition of its synthesis by diacerein and rhein may contribute to the beneficial role of diacerein in OA. Although IL-1 plays a central role in the development and perpetuation of OA, there are close interrelations between factors acting in the disease [58]. The pathophysiology of arthrosis is still not clearly elucidated due to the high complexity of the regulations involved in joint homeostasis. However the inhibition of one or more of these factors involved in cartilage breakdown, could decrease the lesions of cartilage, bone and

synovial membrane, as is shown by diacerein in the experimental animal models of OA [26–28]. The effects of rhein and diacerein observed in our experiments were at concentrations devoid of cell toxicity and close to human therapeutic doses. In humans, the maximum plasma concentration of diacerein was 11 μM after a single dose of 50 mg diacerein [59]. Following a daily administration of 50 mg diacerein every 12 hours for one month, rhein was found in the synovial fluid at concentrations in the 1–10 μM plasma range (personal communication). This confirms that the active compound of diacerein, rhein, was used in the present study at levels which demonstrated pharmacological activities in both in-vivo and in-vitro models. In the present study, activity was found for both the prodrug and active metabolite, as has been reported in other in-vitro studies [32, 35, 36]. Whether this is due to in-vitro conversion of diacerein to rhein or to in-vitro biological activity residing in chemical structures common to both molecules is not known.

In conclusion, diacerein and rhein significantly inhibit IL-1β production and function in human osteoarthritic synovial membrane and cartilage cultures and therefore may inhibit deleterious effects of IL-1β in OA. Their effects on IL-1ra and NO produced in human articular cartilage may also play a beneficial role in this respect. These pharmacological activities of diacerein, observed at doses devoid of cell toxicity and close to human therapeutic doses, contribute to clarification of the mechanisms of action of diacerein which are directly related to the protection of human osteoarthritic human cartilage.

References

1. Mankin HJ. Clinical features of osteoarthritis. In: Kelley's Textbook of Rheumatology, 3rd Ed. Chapter 81. WB Saunders 1989:1480–500.
2. Smith MD, Triantafillou S, Parker A, Youssef PP, Coleman M. Synovial membrane inflammation and cytokine production in patients with early osteoarthritis. J Rheumatol 1997;24:365–71.
3. Chen BX, Francis MJ, Duthie RB, Bromey L, Osman O. Oxygen free radical in human osteoarthritis. Chin Med J 1989;102:931–3.
4. Barrett AJ, Saklatvala J. Proteinases in joint disease. In: Kelley WN, Harris ED, Ruddy S, Sledge CB, Eds. Textbook of Rheumatology, Chapter 14. WB Saunders 1981:195–209.
5. Dingle JT. Recent studies on the control of joint damage. The contribution of the Strangeways Research Laboratory. Ann Rheum Dis 1979;38: 201–14.
6. Rosenberg LC. Structure and function of proteoglycans. In: McCarthy DS, Ed. Arthritis and Allied Conditions, 9th Ed. Chapter 13. Philadelphia: Lea and Febiger 1979:240–55.
7. Sokoloff L. Factors controlling growth and differentiation in the repair of articular cartilage. J Rheumatol 1983;10:53–4.
8. Schumacher HR, Gordon G, Paul H, Reginato A, Villanueva T, Cherian V, Gibilisco P. Osteoarthritis, crystal deposition and inflammation. Semin Arthritis Rheum 1981;11:116–9.
9. Huskisson EC, Dieppe PA, Tucker AK, Cannell LB. Another look at osteoarthritis. Ann Rheum Dis 1979;38:423–8.
10. Ehrlich GE. Pathogenesis and treatment of osteoarthritis. Compr Therapy 1979;5:36–40.
11. Moskowitz RW. Which comes first: inflammation or osteoarthritis. J Rheumatol 1983;10:57–8.
12. Pelletier JP, Dibattista JA, Roughley P, Mccollum R, Martel-Pelletier J. Cytokines and inflammation in cartilage degradation. Rheum Dis Clin North Am 1993;19:545–68.
13. Lotz M, Blanco FJ, Von Kempis J, Dudler J, Maier R, Villiger PM, Geng Y. Cytokine regulation of chondrocyte functions. J Rheumatol 1995;22: 104–8.
14. Goldring MB, Fukuo K, Birkhead JR, Dudek E, Sandell LJ. Transcriptional suppression by interleukin-1 and interferon-gamma of type II collagen gene expression in human chondrocytes. J Cell Biochem 1994;54:85–99.
15. Van De Loo FAJ, Joosten LAB, Van-Lent PL, Arntz OJ, Van Den Berg WB. Role of interleukin-1, tumor necrosis factor alpha, and interleukin-6 in cartilage proteoglycan metabolism and destruction. Effect of in situ blocking in murine antigen- and zymosan-induced arthritis. Arthritis Rheum 1993;38:164–72.
16. Taskiran D, Stefanovic-Racic M, Georgescu H, Evans C. Nitric oxide mediates suppression of cartilage proteoglycan synthesis by interleukin-1. Biochem Biophys Res Commun 1994;200:142–8.
17. Murphy G, Hembry RM, Reynolds JJ. Characterization of a specific antiserum to rabbit stromelysin and demonstration of the synthesis of collagenase and stromelysin by stimulated rabbit articular chondrocytes. Collagen Rel Res 1986;6:351–64.
18. Campbell IK, Piccoli DS, Hamilton JA. Stimulation of human chondrocyte prostaglandin E_2 production by recombinant human interleukin-1 and tumor necrosis factor. Biochim Biophys Acta 1990;1051:310–8.
19. Knott I, Dieu M, Burton M, Houbion A, Remacle J, Raes M. Induction of cyclooxygenase by interleukin-1: Comparative study between human synovial cells and chondrocytes. J Rheumatol 1994;21:462–6.
20. Kato Y, Nakashima K, Iwamoto M et al. Effects of interleukin-1 on syntheses of alkaline phosphatase, type X collagen, and 1,25-dihydroxyvitamin D3 receptor, and matrix calcification in rabbit chondrocyte cultures. J Clin Invest 1993;92:2323–30.
21. Van Beuningen HM, Van Der Kraan PM, Arntz OJ, Van Den Berg WB. Protection from interleukin-1 induced destruction of articular cartilage by transforming growth factor beta: Studies in anatomically intact cartilage in vitro and in vivo. Ann Rheum Dis 1993;52:185–91.

22. Dayer JM, De Rochemonteix B, Burrus B, Demczuk S, Dinarello CA. Human recombinant interleukin-1 stimulates collagenase and prostaglandin E_2 production by human synovial cells. J Clin Invest 1986;77:645–8.
23. Pujol JP, Loyau G. Interleukin-1 and osteoarthritis. Life Sci 1987;41:1187–98.
24. Stadler J, Stefanovic-Racic M, Billiar TR et al. Articular chondrocytes synthesize nitric oxide in response to cytokines and lipopolysaccharide. J Immunology 1991;147:3915–20.
25. Rediske J, Koehne CF, Zhang B, Lotz M. The inducible production of nitric oxide by articular cell types. Osteoarthritis Cart 1994;2:199–206.
26. Brandt KD, Smith G, Kang SY, Myers S, O'Connor B, Albrecht M. Effects of diacerein in an accelerated canine model of osteoarthritis. Osteoarthritis Cart 1997;5:438–49.
27. Moore AR, Greenslade KJ, Alam CA, Willoughby DA. Effects of diacerein on granuloma induced cartilage breakdown in the mouse. Osteoarthritis Cart 1998;1:19–23.
28. Mazieres B, Berdah L, Thiechart M, Viguier G. Diacerein on a postcontusion model of experimental osteoarthritis in the rabbit (French). Rev Rheum Ed Fr 1993;60:77S–81S.
29. Marcolongo R, Fioravanti A, Adami S, Tozzi E, Mian M, Zampieri A. Efficacy and tolerability of diacerein in the treatment of osteoarthrosis. Curr Ther Res 1988;43:878–87.
30. Nguyen M, Dougados M, Berdah L, Amor B. Diacerein in the treatment of osteoarthritis of the hip. Arthritis Rheum 1994;37:529–36.
31. Dougados M, Nguyen M, Berdah L, Lequesne M, Mazieres B, Vignon E. Méthodes d'évaluation de l'arthrose: à propos de l'étude ECHODIAH. Rev Prat 1996;46:S53–6.
32. Pujol JP. Collagenolytic enzymes and interleukin-1: their role in inflammation and cartilage degradation; the antagonistic effects of diacerein on IL-1 action on cartilage matrix components. Osteoarthritis Cart 1993;1:82.
33. Petrillo M, Montrone F, Ardizzone S, Caruso I, Blanchi Porro G. Endoscopic evaluation of diacerein-induced gastric mucosal lesions. Curr Ther Res 1991;49:10–5.
34. La Villa G, Marra F, Laffi G, Belli B, Meacci E, Fascetti P, Gentilini P. Effects of rhein on renal arachidonic acid metabolism and renal function in patients with congestive heart failure. Eur J Clin Pharmacol 1989;37:1–5.
35. Franchi-Micheli S, Lavacchi L, Friedmann CA, Zilletti L. The influence of diacerein on the biosynthesis of prostaglandin-like substances in vitro. J Pharm Pharmacol 1983;35:262–4.
36. Mian M, Brunelleschi S, Tarli S, Rubino A, Benetti D, Fantozzi R. Rhein: an anthraquinone that modulates superoxide anion production from human neutrophils. J Pharm Pharmacol 1987; 39:845–7.
37. Mian M, Benetti D, Rosini S, Fantozzi R. Effects of diacerein on the quantity and phagocytic activity of thioglycollate-elicited mouse peritoneal macrophages. Pharmacology 1989;39:362–6.
38. Yaron I, Meyer FA, Dayer JM, Bleiberg I, Yaron M. Some recombinant human cytokines stimulate glycosaminoglycan synthesis in human synovial fibroblast cultures and inhibit it in human articular cartilage cultures. Arthritis Rheum 1989; 32:173–80.
39. Castor CW, Bignall MC, Hassler PA, Roberts DJ. Connective tissue activation. XXI: Regulation of glycosaminoglycan metabolism by lymphocyte (CTAP-I) and platelet (CTAP-III) growth factors. In Vitro Cell Dev Biol 1981;17:777–85.
40. Ashab I, Peer G, Blum M, Wollman Y, Chernihovsky T, Hassner A, Schwartz D, Cabili S, Silverberg D, Iaina A. Oral administration of L-arginine and captopril in rats prevents chronic renal failure by nitric oxide production. Kidney Int 1995;47: 1515–21.
41. Pelletier JP, Martel-Pelletier J. Evidence for the involvement of interleukin-1 in human osteoarthritic cartilage degradation: Protective effect of NSAID. J Rheumatol 1989;16:19–27.
42. Pelletier JP, Faure MP, Dibattista JA, Wilhelm S, Visco D, Martel-Pelletier J. Coordinate synthesis of stromelysin, interleukin-1, and oncogene proteins in experimental osteoarthritis. An immunohistochemical study. Am J Pathol 1993;142:95–105.
43. Campbell IK, Piccoli DS, Butler DM, Singleton DK, Hamilton JA. Recombinant human interleukin-1 stimulates human articular cartilage to undergo resorption and human chondrocytes to produce both tissue and urokinase type plasminogen activator. Biochim Biophys Acta 1988;967:183–94.
44. Morrison DC, Ryan JL. Endotoxins and disease mechanisms. Ann Rev Med 1987;38:417–32.
45. Bendrups A, Hilton A, Meager A, Hamilton JA. Reduction of tumor necrosis factor alpha and interleukin-1 beta levels in human synovial tissue by interleukin-4 and lucocorticoid. Rheumatol Int 1993;12:217–20.
46. Martel-Pelletier J, Mineau F, Jolicoeur FC, Cloutier JM, Pelletier JP. In vitro effects of diacerein and rhein on IL-1 and TNF-α systems in human osteoarthritic synovium and chondrocytes. J Rheumatol 1998;25:753–62.
47. Smith RJ, Chin JE, Sam LM, Justen JM. Bieffects of an interleukin-1 receptor antagonist protein on interleukin-1-stimulated cartilage erosion and chondrocyte responsiveness. Arthritis Rheum 1991;34:78–83.
48. Matsukawa A, Ohkawara S, Maeda T, Takagi K, Yoshinaga M. Production of IL-1 and IL-1 receptor antagonist and the pathological significance in lipopolysaccharide-induced arthritis in rabbits. Clin Exp Immunol 1993;93:206–11.
49. Caron JP, Fernandes JC, Martel-Pelletier J, Tardif G, Mineau F, Geng C, Pelletier JP. Chondroprotective effect of intraarticular injections of interleukin-1 receptor antagonist in experimental osteoarthritis. Arthritis Rheum 1996;39:1535–44.
50. Pelletier JP, Howell DS. Etiopathogenesis of osteoarthritis. In: McCarthy DJ, Koopman WJ, Eds. Arthritis and Allied Conditions, Ed. 12 (A Textbook of Rheumatology, Vol. 2). Philadelphia: Lea and Febiger 1993:1723–34.
51. Cruz TF, Tang J, Pujol J-P. Mechanisms involved in diacerein inhibition of collagenase expression. Rev Prat 1996;46:515–9.
52. Dingle JT, Horner A, Shield M. The sensitivity of synthesis of human cartilage matrix to inhibition by IL-1 suggests a mechanism for the development

of osteoarthritis. Cell Biochem Funct 1991;9: 99–102.
53. Farrell AJ, Blake DR, Palmer RM, Moncada S. Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. Ann Rheum Dis 1992;51:1219–22.
54. Stefanovic-Racic M, Stadler J, Georgescu HI, Evans CH. Nitric oxide synthesis and its regulation by rabbit synoviocytes. J Rheumatol 1994;21:1892–8.
55. Stefanovic-Racic M, Stadler J, Georgescu HI, Evans CH. Nitric oxide and energy production in articular chondrocytes. J Cell Physiol 1994;159:274–80.
56. Palmer RM, Hickery MS, Charles IG, Moncada S, Bayliss MT. Induction of nitric oxide synthase in human chondrocytes. Biochem Biophys Res Commun 1993;193:398–405.
57. Pelletier JP, Mineau F, Ranger P, Tardif G, Martel-Pelletier, J. The increased synthesis of inducible nitric oxide inhibits IL-1ra synthesis by human articular chondrocytes: possible role in osteoarthritic cartilage degradation. Osteoarthritis Cart 1996;4:77–84.
58. Henrotin YE, De Groote DD, Labasse AH, Gaspar SE, Zheng SX, Geenen VG, Reginster JY. Effects of exogenous IL-1β, TNF-α, IL-6, IL-8 and LIF on cytokine production by human articular chondrocytes. Osteoarthritis Cart 1996;4:163–73.
59. Debord P, Louchahi K, Tod M, Cournot A, Perret G, Petitjean O. Influence of renal function on the pharmacokinetics of diacerein after a single oral dose. Eur J Drug Metab Pharmacokinet 1994; 19:13–9.