Down regulation of interleukin 1β production in human osteoarthritic synovial tissue and cartilage cultures by aminoguanidine

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Abstract
***Objective*—To evaluate the effect of aminoguanidine (AG) on de novo interleukin 1β (IL1β), nitric oxide (NO), and interleukin 1 receptor antagonist (IL1ra) production by osteoarthritic human synovial tissue and articular cartilage cultures.**
***Methods*—Synovial tissue and cartilage, obtained during surgery from 29 patients undergoing total knee or hip replacement for osteoarthritis, were cut into small pieces and cultured in the presence or absence of lipopolysaccharide (LPS) and test materials. IL1β, IL1ra, and NO were determined in culture media. The inducible nitric oxide synthase inhibitor, AG, was added to cultures in various concentrations (0.3–3 mmol/l).**
***Results*—In synovial tissue cultures AG (0.3, 1, and 3 mmol/l) decreased LPS (1 μg/ml) stimulated IL1β and NO release in the media in a dose dependent manner ($p<0.05$ at 1 mmol/l and $p<0.05$ at 0.3 mmol/l, respectively). In articular cartilage cultures AG (0.3, 1, and 3 mmol/l) decreased LPS (1 μg/ml) stimulated IL1β and NO release in the media in a dose dependent manner ($p<0.05$ at 1 mmol/l and $p<0.01$ at 0.3 mmol/l, respectively). Hydrocortisone (5 μg/ml) also significantly decreased LPS stimulated IL1β release in media of synovial tissue and cartilage cultures and NO in media of synovial cultures. AG (0.3, 1, and 3 mmol/l) decreased LPS (1 μg/ml) stimulated IL1ra levels in media of synovial tissue cultures in a dose dependent manner ($p<0.05$ at 1 mmol/l) but increased LPS (1 μg/ml) stimulated IL1ra release in media of cartilage cultures ($p<0.01$ at 3 mmol/l). The NO donor, nitroprusside (10, 30, 100, and 300 μg/ml) stimulated IL1β release in media of synovial tissue cultures in a dose dependent manner ($p<0.01$ at 100 μg/ml). AG and nitroprusside at the concentrations used had no toxic effect on human synovial cells.**
***Conclusions*—NO synthase inhibitors may modulate osteoarthritis and articular inflammatory processes not only by decreasing NO synthesis but also by their effects on ILβ and IL1ra production.**
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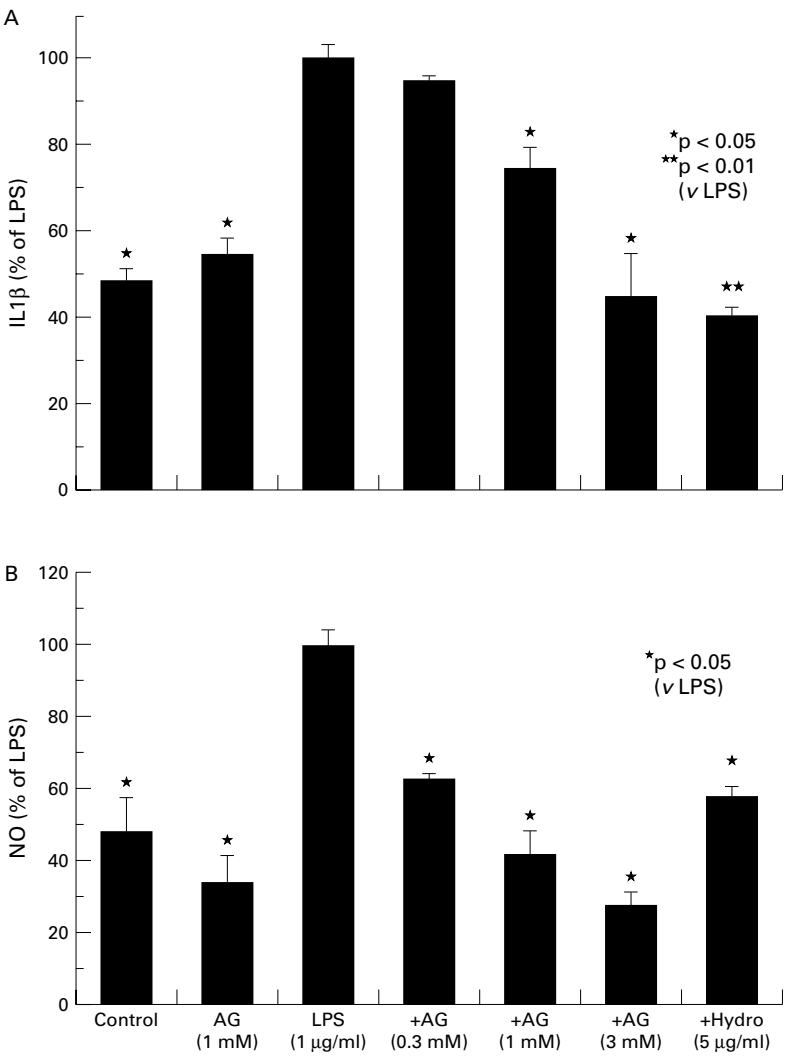

Figure 1 Effect of aminoguanidine (AG) and hydrocortisone (Hydro) on the release of interleukin 1β (IL1β) and nitric oxide (NO) in the media of lipopolysaccharide (LPS) stimulated synovial tissue cultures. Bars show the mean and SEM of three separate experiments. The absolute mean (SEM) levels of IL1β in control and in LPS stimulated cultures were 7.3 (0.89) and 14.9 (2.5) pg/mg synovia, respectively. The absolute mean (SEM) levels of NO in control and LPS stimulated cultures were 2.13 (0.2) and 4.34 (0.19) μmol/mg synovial tissue, respectively.

Cytokines have important roles in cartilage destruction in osteoarthritis (OA).[1 2] Among them, it appears that interleukin 1 (IL1) has a key role[3] and nitric oxide (NO) also plays a part.[4] Both IL1 and NO contribute to cartilage degradation and inhibition of its synthesis.[5 6] Because IL1 was shown to stimulate NO production in osteoarthritic synovium and cartilage,[7 8] it has been suggested that the deleterious effects of IL1 on articular cartilage are mediated, at least in part, by NO.[9 10] Therefore, inhibitors of NO synthesis are being investigated as possible anti-arthritic agents.[11] Aminoguanidine (AG) is a relatively selective inducible nitric oxide synthase inhibitor.[12 13] Lipopolysaccharide (LPS) is arthritogenic in animals[14] and an inducer of IL1 and NO in human synovial and cartilage cultures.[15] This study aimed at evaluating the effect of AG on

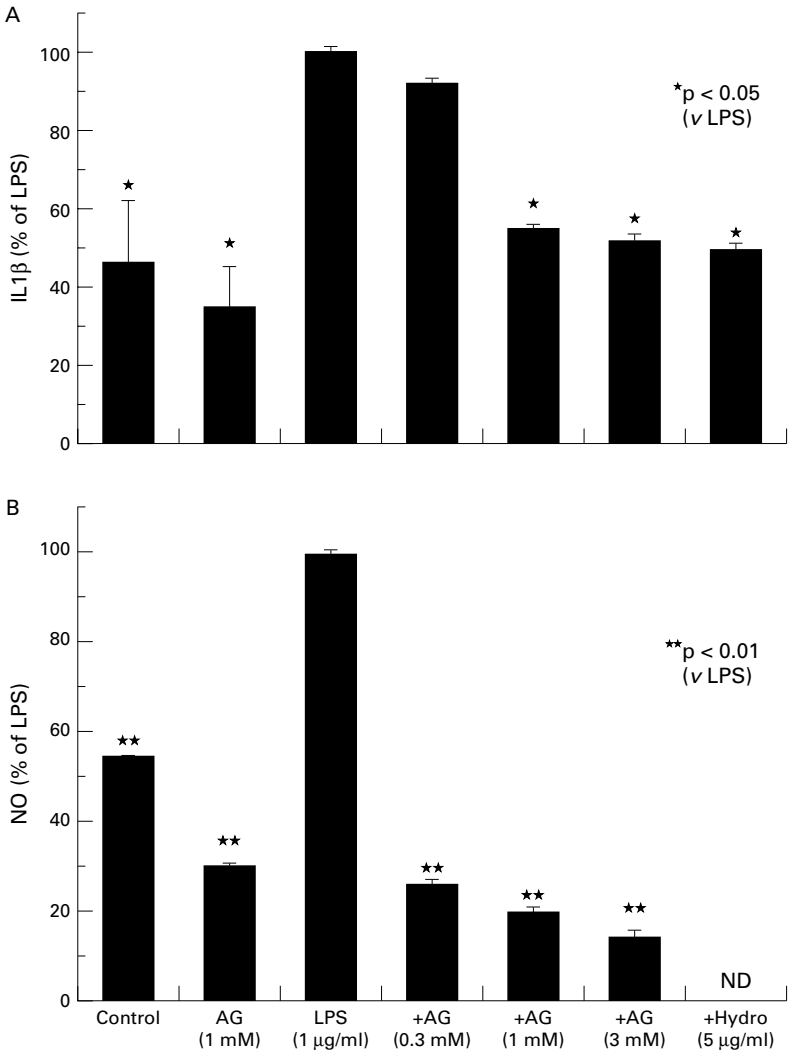

Figure 2 Effect of aminoguanidine (AG) on the release of interleukin 1β (IL1β) and nitric oxide (NO) in the media of lipopolysaccharide (LPS) stimulated cartilage cultures. Bars show the mean and SEM of six separate experiments. The absolute mean (SEM) levels of IL1β in control and LPS stimulated cultures were 0.29 (0.01) and 0.64 (0.12) pg/mg cartilage, respectively. The absolute mean (SEM) levels of NO in control and LPS stimulated cultures were 15.8 (0.001) and 30.68 (0.003) μmol/mg cartilage, respectively.

de novo IL1β and NO production in human synovial and cartilage cultures. We presume that in vivo certain factors can stimulate IL1β and NO production in osteoarthritic joints, as LPS does in vitro in a non-specific manner.[16]

We describe inhibition of IL1β production by AG in human osteoarthritic synovial tissue and cartilage cultures in the presence of LPS and stimulation of IL1β production by a NO donor (nitroprusside), suggesting that NO and its inhibitors may modulate IL1β production in articular tissues.

Materials and methods

AG was from Sigma (St Louis, MO, USA), nitroprusside from Schwarz Pharma (Monheim, Germany), LPS from Difco Laboratories (Detroit, MI, USA), and IL1β from Biogen SA (Geneva, Switzerland).

SPECIMEN SELECTION AND CULTURE CONDITIONS

Synovial tissue and cartilage were obtained during surgery from 29 patients undergoing total knee or hip replacement owing to OA (21 women, eight men, mean (SD) age 69.4 (7.5) and 73.2 (3.5) years, respectively).

SYNOVIAL TISSUE CULTURES

Within two hours of removal, synovial tissue was cut into small pieces (a few millimetres in diameter) and cultured in 2 cm Petri dishes with test materials in RPMI 1640 supplemented with L-glutamine (2 mmol/l), penicillin (100 U/ml), and streptomycin sulphate (100 μg/ml). After 48 hours of incubation (37°C, 5% CO_2), NO and IL1β were measured in culture media.

CARTILAGE CULTURES

Cartilage tissue was finely diced, randomised, and distributed uniformly in 96-well plates containing 0.2 ml Dulbecco's modified Eagle's medium, 1% fetal calf serum, penicillin, and streptomycin.

NO, IL1β AND IL1ra DETERMINATIONS

NO synthesis was determined as previously described by Ashab *et al.*[17] NO_2 and NO_3 were determined after the reduction of NO_3 to NO_2 by a 90 minute incubation in a tilting bath (37°C) using nitrate reductase from *Escherichia coli* and β-nicotinamide adenine dinucleotide phosphate (reduced form) (Sigma) as cofactor. The presence of NO_2 was determined with the Griess reagent. Sensitivity of the procedure was 3 μmol/l. IL1β and interleukin 1 receptor antagonist (IL1ra) in culture media were determined by Quantikine Human Immunoassay (R&D Systems Inc, USA), which employs the quantitative sandwich enzyme immunoassay technique.

Cell viability and toxicity in the presence of AG and nitroprusside, incubated with target cells for 72 hours, were determined in human synovial monolayer cultures by trypan blue exclusion test and by the tetrazolium salt XTT assay.[18]

Statistical significance (determination of p values) was evaluated by analysis of variance and by Student's *t* test. p Values <0.05 were considered significant.

Individual experiments were performed in triplicate or quadruplicate. IL1β and NO were determined per milligram of tissue. Results, reported as the percentage of stimulated cultures, represent the findings from several (at least three) experiments. The number of experiments performed and the absolute values for controls and stimulated cultures are reported in the figure legends.

Values for IL1β and NO in figs 1 and 2 were obtained in media of the same experiments.

Results

SYNOVIAL TISSUE CULTURES

LPS (1 μg/ml) stimulated synovial IL1β and NO release into tissue culture media twofold (fig 1). AG (0.3, 1, and 3 mmol/l) decreased stimulated IL1β release in a dose dependent manner by 5%, 26%, and 56%, respectively, and NO release by 40%, 62%, and 100%, respectively. Hydrocortisone (5 μg/ml) also decreased stimulated IL1β and NO release by 58% and 45%, respectively. In the absence of LPS stimulation, AG alone (1 mmol/l) had no significant effect on IL1β and NO release as compared with the control (fig 1).

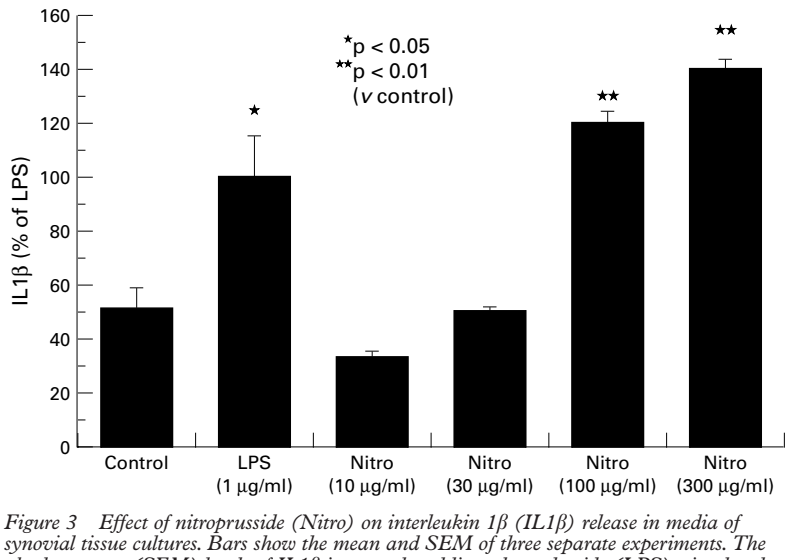

Figure 3 Effect of nitroprusside (Nitro) on interleukin 1β (IL1β) release in media of synovial tissue cultures. Bars show the mean and SEM of three separate experiments. The absolute mean (SEM) levels of IL1β in control and lipopolysaccharide (LPS) stimulated cultures were 7.46 (3.69) and 13.69 (4.5) pg/mg synovia, respectively.

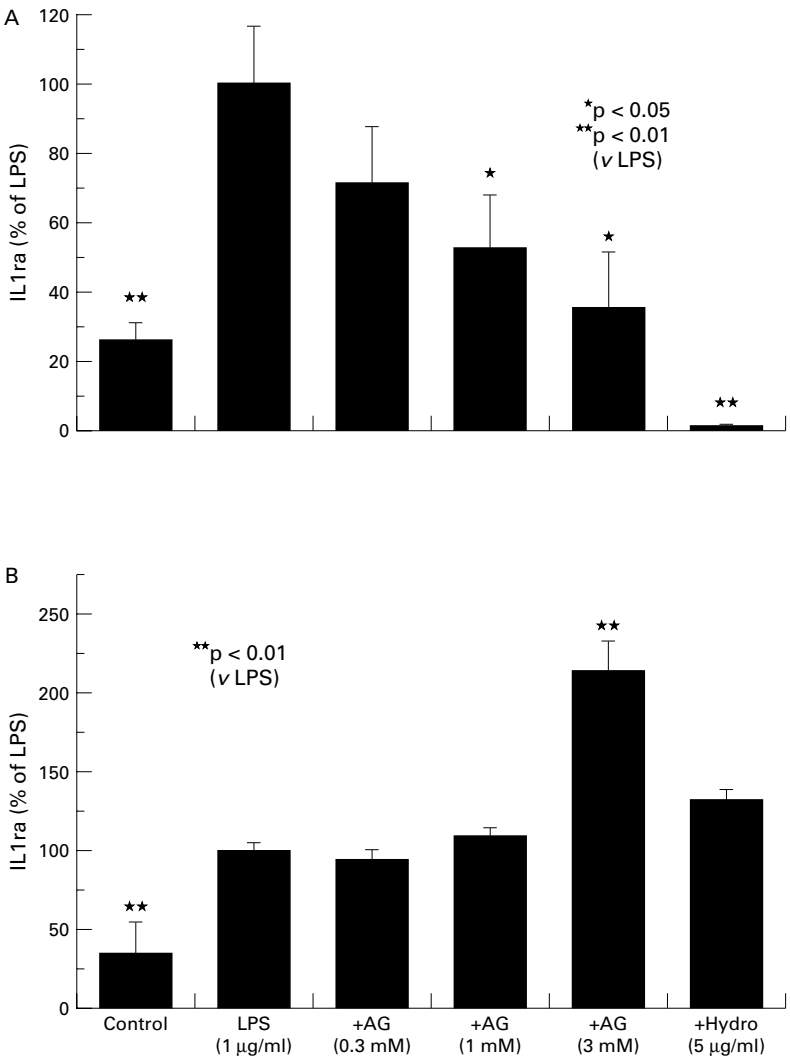

Figure 4 Effect of aminoguanidine (AG) and hydrocortisone (Hydro) on the release of interleukin 1 receptor antagonist (IL1ra) in lipopolysaccharide (LPS) stimulated synovial tissue (A) and cartilage (B) cultures. Bars show the mean and SEM of three separate experiments. The absolute mean (SEM) levels of IL1ra in control and LPS stimulated cultures were 163.5 (29.84) and 651 (107) pg/mg synovia, respectively, and for cartilage cultures 1.4 (0.2) and 4.5 (0.24) pg/mg cartilage, respectively.

The NO donor, nitroprusside (10, 30, 100, and 300 μg/ml), stimulated IL1β release in media of synovial tissue cultures in a dose dependent manner, reaching statistical significance at 100 μg/ml nitroprusside (more than twofold stimulation) (fig 3). Under the same conditions LPS (1 μg/ml) stimulated IL1β production twofold (fig 3). AG (0.3, 1, and 3 mmol/l) decreased LPS stimulated IL1ra levels in the media of synovial tissue cultures in a dose dependent manner (fig 4A). Hydrocortisone (5 μg/ml) completely abrogated IL1ra release in synovial tissue media (fig 4A).

CARTILAGE CULTURES

LPS (1 μg/ml) stimulated IL1β and NO release in media of cartilage cultures about twofold (fig 2). AG (0.3, 1, and 3 mmol/l) decreased LPS stimulated IL1β and NO release in a dose dependent manner ($p<0.05$ at 1 mmol/l and $p<0.01$ at 0.3 mmol/l, respectively). Hydrocortisone (5 μg/ml) also significantly decreased stimulated IL1β levels (fig 2). Nitroprusside (30 μg/ml), on the other hand, stimulated IL1β production in cartilage cultures threefold (data not shown). AG (3 mmol/l) significantly increased IL1ra release in media of LPS stimulated cartilage cultures ($p<0.01$) (fig 4B).

Neither AG (0.3, 1, and 3 mmol/l) nor nitroprusside (10, 30, 100, and 300 μg/ml) had any toxic effects, nor did they affect the viability of human synoviocytes (data not shown).

Discussion

AG, a nitric oxide inhibitor, significantly decreased LPS stimulated levels of IL1β in media of osteoarthritic synovial tissue and cartilage cultures (figs 1 and 2). This observation suggests that IL1β synthesis in some articular tissues may be mediated, at least in part, by NO. Indeed, the NO donor, nitroprusside, significantly stimulated IL1β production by osteoarthritic synovial tissue and cartilage cultures, further supporting the notion that NO may regulate IL1β production in osteoarthritic articular tissues. McInnes *et al* have shown that the NO donor s-nitrosoacetylpenicillamine produced high concentrations of tumour necrosis factor α in rheumatoid synoviocytes and macrophage cell cultures.[19] Others have found inhibition of IL1 and prostaglandin E levels in synovial fluids of LNAME treated rabbits with antigen induced arthritis.[20] Pelletier *et al* have shown that a selective inhibitor of inducible NO synthase, L-NIL, reduced progression of experimental OA in dogs.[11] Within the same context they noticed and reported that L-NIL reduces the production of IL1β by the osteoarthritic canine synovial tissue. In view of our present results and the above mentioned observations, it might be appropriate to suggest a mechanism for the pathogenesis of OA wherein IL1 and NO reciprocally affect the synthesis of each other and each has its own effect on cartilage destruction.

This hypothetical chain of events may explain why NO synthase inhibitors only partially reverse LPS and IL1β induced inhibition of [^{35}S]glycosaminoglycan synthesis by articular cartilage cultures.[21] IL1β is not completely dependent on NO for its inhibitory action on cartilage matrix synthesis,[8] therefore inhibiting NO synthesis only partially reverses

this process. Others have described alleviation of IL1 induced proteoglycan synthesis suppression in animal articular cartilage by the NO synthase inhibitor, n(G)-monomethyl-L-arginine.[9] Van de Loo *et al* suggest that inhibition of proteoglycan synthesis may occur through different pathways.[7] The value of the possible use of NO synthase inhibitors for arthritis is under consideration.[22] NO can inhibit articular cartilage synthesis[20] and activate metalloprotease enzymes in articular cartilage.[5] Thus, from this point of view, the use of NO synthase inhibitors may be beneficial for destructive processes occurring in OA[4 7 11] and rat adjuvant arthritis.[23 24] However, the group of Evans *et al* have repeatedly drawn attention to the fact that NO synthase inhibitors may be only weakly therapeutic, or even detrimental, in established disease.[22 24 25] They call for caution when trying to evaluate NO involvement in the pathophysiology of arthritis, which can be complex, and note that NO inhibitors counteract suppression of IL1 induced glycosaminoglycan synthesis but may exacerbate proteoglycan catabolism.[26] An editorial by St Clair focuses on the dilemma of the role played by NO in arthritis.[27] There is less of a dilemma on the issue of the pivotal detrimental role played by IL1 in articular cartilage destruction.[3] Therefore, there is a natural interest in IL1 inhibitors for the management of OA.[15] A way of inhibiting IL1 deleterious effects on articular cartilage may be by increasing levels of IL1ra in the milieu. Thus it was suggested that human IL1ra gene transfer into human synovial fibroblasts is chondroprotective.[28] In this context our findings on the effects of NO inhibitors on IL1ra produced by synovial tissue and human articular cartilage are of interest but, in a way, confusing. While AG decreases levels of LPS stimulated IL1ra in media of synovial tissue cultures, it produces an increase in media of cartilage cultures (fig 4). Possibly, the local increase of IL1ra in cartilage is more important for chondroprotection than the decrease in synovial tissue production, but this matter is still open for debate. The Pelletier group has shown that increased synthesis of inducible NO inhibits IL1ra synthesis by human articular chondrocytes.[29] However, the exact mechanism by which NO inhibitors modulate IL1β and IL1ra synthesis remains unclear and requires further study.

In summary, we suggest that some NO synthase inhibitors may affect cartilage homeostasis not only by their inhibitory effect on de novo NO production in articular tissues but also by their effects on de novo IL1β and IL1ra secretion in these tissues.

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