Effects of dexamethasone on proteoglycan content and gene expression of IL-1ß-stimulated osteoarthrotic chondrocytes in vitro

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ABSTRACT – We studied the effects of dexamethasone on proteoglycan (PG) concentration and gene expression in human osteoarthrotic chondrocytes stimulated with IL-1ß.

Cartilage samples were taken from 7 patients with osteoarthrosis of the knee and chondrocytes cultivated in alginate beads. Dexamethasone was added in three concentrations (10^{-5}, 10^{-6}, 10^{-7}M) to IL-1ß (100 pg/mL)-stimulated chondrocytes. PG concentration was estimated by a dimethylmethylene blue assay. To assess cell proliferation, DNA content was measured fluorometrically. Quantitative Lightcycler-PCR was used to estimate the mRNA levels of stromelysin-1 (MMP-3) and aggrecan (AGG).

The proliferation rate was unchanged in all treatment groups. IL-1ß increased MMP-3 expression by 44% and inhibited AGG expression by 16%, but PG-concentration was reduced by 7%. The addition of dexamethasone to IL-1ß-stimulated chondrocytes further reduced the PG concentration by 19% at 10^{-5}M and by 17% at 10^{-7}M. The MMP-3 expression was inhibited between 27–53% and the AGG expression between 30–46% by dexamethasone.

In osteoarthrotic chondrocytes, dexamethasone in an appropriate dose range reduced the expression of MMP-3 and AGG at the same time. The resulting decrease in PG concentration should be considered when using intraarticular corticosteroids to treat an osteoarthrotic joint.

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Intraarticular injections with corticosteroids, such as dexamethasone, have been recommended for patients suffering from osteoarthrosis who have an effusion and signs of inflammation (Caldwell 1996). Dexamethasone has been shown to inhibit the synthesis of inflammatory mediators in the affected joint (DiBattista et al. 1993). Beside their inhibitory effects on key proteins, as exemplified by MMPs, such as collagenase and stromelysin (Yang-Yen et al. 1990), steroids have also been shown to suppress cytokines, such as IL-1 (Larrick and Kunkel 1988). Similarly, the corticosteroids suppress the synthesis of major cartilage matrix components, such as collagen type II and proteoglycan (Pelletier et al. 1989, Farquhar et al. 1996, Nakamura et al. 1997, MacLeod et al. 1998). However, the complex effect of dexamethasone on the anabolic and catabolic metabolism of chondrocytes is still disputed (Guerne et al. 1999, Saito et al. 1999, Miyazaki et al. 2000), despite their use in osteoarthrosis during the past 4 decades (Caldwell 1996). This controversy, partly caused by the fact that although steroids are very effective in treating joint inflammation and pain, they may accelerate cartilage breakdown (Caldwell 1996), has led to studies trying to correlate their effects with the concentrations used (Pelletier et al. 1989, Saito et al. 1999). It is of interest that the concentration of corticosteroids needed to suppress proteoglycan synthesis is higher than that needed to suppress metalloproteinase synthesis (Pelletier and Martel-

Pelletier 1989a). When used in a low dose, it was shown that corticosteroids effectively reduce OA changes (Pelletier and Martel-Pelletier 1989b), but in higher concentrations of dexamethasone, it causes deterioration of cartilage (Behrens et al. 1975).

Despite the long use of intraarticular dexamethasone, most studies are done with nonhuman cartilage, but little is known about the effects on chondrocytes originating from a patient group eligible for treatment with corticosteroids. Therefore, we analyzed the effects of dexamethasone on PG metabolism and the gene expression of aggrecan (AGG) and stromelysin-1 (MMP-3) which are major cartilage metabolites. MMP-3 represents an important metalloproteinase in the degradation process of cartilage (Ganu et al. 1994), and AGG is the major proteoglycan in cartilage tissue (Bolton et al. 1996).

Material and methods

Cell preparation and cultivation

We harvested human chondrocytes from 7 patients (mean age 71 (61–79) years, 4 women) who underwent total knee replacement for advanced knee osteoarthrosis. All patients had grades II-IV osteoarthrotic changes as assessed by the Kellgren and Lawrence (1957) scoring system. Macroscopically, the cartilage showed surface irregularities with fibrillation and fissuring partly down to the subchondral bone.

To isolate chondrocytes, the cartilage was peeled from the resected femur and tibia, after which the pieces of cartilage were digested with 0.4% pronase and 0.02% collagenase (12 mL/g cartilage) (Boehringer Mannheim, Germany) at 37 °C overnight. The chondrocytes released were rinsed, filtered and counted, and then resuspended in 1.2% alginate (Keltone LV, Kelco, Chicago, USA). By placing the chondrocyte suspension in isoosmotic calcium chloride solution (102 mM), alginate beads containing about 4×10^4 chondrocytes were formed. These beads were incubated at 37 °C, 95% humidity and 5% CO_2. The culture medium, containing 45% Ham's medium F12, 45% DMEM (Biochrom seromed, Berlin, Germany), 10% fetal bovine serum (FBS) (Biochrom seromed), ciprofloxacin (Bayer, Leverkusen, Germany), ascorbic acid 10 µg/mL and glutamate 2 mM, was changed daily. After 3 days, the cultures were divided into 5 treatment groups: 1) IL-1ß (100 pg/mL; Genzyme, Cambridge, USA), 2) IL-1ß (100 pg/mL) with dexamethasone at 10^{-5}M, 3) Il-1ß (100 pg/mL) with dexamethasone at 10^{-6}M, 4) Il-1ß (100 pg/mL) with dexamethasone at 10^{-7}M, and 5) control. All cultures were treated with fresh medium with/without supplement every other day for 1 week. At the end of the experiment, the beads were dissolved in sodium citrate solution (55 mM sodium citrate, 30 mM EDTA, 90 mM sodium chloride, pH 7.45.

Dimethylmethylene blue assay for proteoglycan content

We determined the PG concentration (µg/mL) by binding to dimethylmethylene blue (DMB) dye (16 mg/mL DMB, 0.03M sodium formate, 0.2% formic acid, pH 6.8) in the presence of 0.24M GuHCl, since this prevents DNA precipitation (Chandrasekhar et al. 1987). Absorbency was measured at 530 and 595nm using the plate reader MRX from Dynatech (Medical Products, Guernsey, Channel Islands, UK). Modifications of the method included the adaptation of the dilution buffer to the alginate system and the use of papain-digested probes. The results are reported as equivalents of a standard of purified bovine nasal aggrecan.

DNA content

To normalize the various chondrocyte cultures for cell number, we measured total DNA content in the aliquots of sodium citrate dissolved beads after digesting cells with papain solution (Häuselmann et al. 1992). DNA content (µg/mL) was determined with a modified fluorometric assay (emission measured at 400–550 nm for an excitation wavelength of 365 nm), using bisbenzididazole dye (Hoechst dye 33258; Polysciences, Warrington, USA).

Lightcycler PCR

Total RNA from cultured cells was prepared, using the acid guanidinium-thiocyanate phenol-chloroform method (Chomczynski and Mackey 1995), and its concentration measured with a spectrophotometer. For reverse transcription we used oligodT primer and 1 µg total RNA (cDNA kit; Roche, Mannheim, Germany). For quantification of cDNA with Lightcycler-PCR (LC-PCR) the following primers were used: MMP-3 (left 5'-GGG CCCT CAA GGA AAA GAA TC-3'; right 5'-CAG TTG GGC ATT GGT GTA GA-3'), AGG (left 5'-TGA GGC CAG CAG AGA AGA TT-3', right 5'-TCT CCG CTG ATT TCA GTC CT-3'). To compare individual gene expression of chondrocyte cultures we used GAPDH (glyceraldehyde-3-phosphate dehydrogenase) as household gene (primer design: left 5' - GAG TCC ACT GGC GTC TTC AC - 3'; right 5' - GGT GCT AAG CAG TTG GTG GT - 3'). For LC-PCR of MMP-3, AGG and GAPDH standards were established. In short, standards were generated by inserting cDNA (amplified with above-mentioned primer) into the cloning site of pCR4 TOPO (TOPO TA Cloning Kit, Invitrogen, Groningen, The Netherlands). For each standard, a dilution row was made. Parallel to the amplification of the probes, 4 dilutions of the known standards were amplified (LC-PCR protocol: denaturation 1 minute at 95 °C; annealing 7.4–10.1 sec at 60 °C followed by elongation for 15 seconds at 72 °C). For analysis of data, we used LightCycler Software Version 3.39 (Roche, Basel, Switzerland). Results are given in % of expression of MMP-3 and AGG relative to GAPDH expression.

Statistics

Results are expressed as the mean (standard error of the mean). Statistical comparisons of the means (PG/DNA concentration) were done using multivariate analysis of variance (ANOVA) because of similar variances of the data. In the case of multiple testing, Tukey adjustment was applied. Matched data (LC-PCR data) were analyzed using Wilcoxon's signed ranks test.

Results

Effects of IL-1ß and dexamethasone on DNA content

The addition of IL-1ß and dexamethasone to the various concentrations of osteoarthrotic chondrocytes cultured in alginate beads had no effect on the DNA content, as compared to the control.

Gene expression of stromelysin-1 and aggrecan in chondrocyte cultures after treatment with various concentrations of dexamethasone (n = 6) expressed as the mean percentage (standard error of the mean) of relative expression of GAPDH, compared to control without dexamethasone set at 100%

	Dexamethasone concentration		
	10^{-7} M	10^{-6} M	10^{-5} M
Stromelysin-1	70.5 (5.8)	55.1 (8.8)	43.8 (8.5)
Aggrecan	70.0 (10.8)	63.9 (11.1)	54.2 (10.0)

All chondrocyte cultures were stimulated with IL-1ß (100 pg/mL). Quantitative PCR (Lightcycler PCR) was done to estimate expression levels of stromelysin-1 and aggrecan.
The reduction in stromelysin-1 and aggrecan gene expression, as compared to the control was statistically significant (p = 0.04).

Effects of IL-1ß on chondrocyte metabolism

Treatment with IL-1ß (100 pg/mL) reduced the PG/DNA concentration in chondrocyte cultures, as compared to the untreated controls. The PG/DNA concentration decreased from 28.57 (3.63) to 26.61(3.50) (p = 0.05).

It had opposite effects on AGG and MMP-3 gene expression. AGG expression was diminished by 16% (p = 0.04) and MMP-3 expression enhanced by 44% (p = 0.04) of the controls.

Effects of dexamethasone on proteoglycan content

The addition of dexamethasone to IL-1ß-stimulated chondrocyte cultures reduced the PG/DNA concentration, still more, and in all three concentrations. The highest concentration (10^{-5}M) reduced the PG/DNA concentration to 22.36 (3.25) ($p < 0.0001$), the lower concentrations (10^{-6} and 10^{-7}) reduced the PG to 22.69 (2.90) ($p < 0.0001$) and to 22.79 (2.76) ($p < 0.0001$), respectively.

AGG and MMP-3 gene expression after dexamethasone treatment

Unlike the regulation of gene expression by IL-1ß alone, we found a comparable downregulation of both MMP-3 and AGG (Table) by dexamethasone. The reductions in MMP-3 and AGG expression, as compared to the control, were statistically significant (p = 0.04). The suppressive effects of various dexamethasone concentrations on both gene expression levels were not statistically significant among one another.

Discussion

In this culture model of IL-1ß-stimulated chondrocytes, dexamethasone inhibited both the gene expression of the matrix degrading metalloproteinase MMP-3 and of aggrecan, an important structural cartilage component. In addition, dexamethasone treatment reduced the PG concentration by 15% at different concentrations.

The short-term benefit of intraarticular corticosteroids in patients with osteoarthrosis has been questioned in the light of its potential long-term deleterious effects on the cartilage. Previous animal studies have shown that the effects of corticosteroids are closely related to their dosage and the intervals between the treatments (Pelletier and Martel-Pelletier 1989b).

In this study, we used dexamethasone concentrations ranging from a low dose of 10^{-7} M to a higher one of 10^{-5} M, since it has been well established that dexamethasone inhibits MMP activity at its maximum within this range (DiBattista et al. 1993). Others have suggested (Pelletier et al. 1989, Pelletier and Martel-Pelletier 1989a) that the administration of low dose steroids may suppress catabolism sufficiently without significantly affecting the anabolism of cartilage. In our culture model, we found no difference in the regulation of proteoglycans and metalloproteinases at the level of gene expression. In a dog model, treatment with low doses of corticosteroids reduced osteophyte formation and cartilage lesions (Pelletier and Martel-Pelletier 1989b). These effects were ascribed to a dose-dependent effect of corticosteroids on cartilage anabolism and catabolism. Low doses of corticosteroids with tissue concentrations of 10^{-7} M are supposed to suppress catabolism, but have no harmful effect on anabolism (Pelletier and Martel-Pelletier 1989b).

Our results are consistent with those of previous studies showing a downregulation by dexamethasone of various MMPs, including MMP-3 (Pelletier et al. 1989, Yang-Yen et al. 1990, Delany and Brinckerhoff 1992). The downregulation of degradative metalloproteinases even at very low concentrations together with a suppression of the activators of MMPs (Hamilton et al. 1981, Larrick and Kunkel 1988) may be important mechanisms for suppressing catabolism. It is noteworthy that recent studies (Su et al. 1996) showed that dexamethasone can inhibit the physiological inhibitors of MMPs (TIMP). The clinical implications of this downregulation of TIMP together with the downregulation of type II collagen expression (Miyazaki et al. 2000) need further elucidation.

Our results clearly show that the effects of dexamethasone on AGG gene expression are comparable to those on MMP-3. AGG is the major proteoglycan in cartilage matrix and it is responsible for the swelling pressure in the collagen network. To determine whether changes in the gene expression of the quantitatively most important proteoglycan of articular cartilage affect the PG concentration, we analyzed the PG/DNA concentration in alginate beads. It should be noted that the PG concentration may be influenced by both a change in the metabolism of AGG and MMP-3 as well. In our experiments, the inhibition of AGG and MMP-3 expression caused a reduction in the PG concentration. This was even true for the lowest dexamethasone concentration used. This finding is consistent with previous studies of glucocorticosteroids on the PG release in cartilage explant cultures stimulated with IL-1ß (Arsenis and McDonnell 1989, Augustine and Oleksyszyn 1997). No protective effect on cartilage degradation was detected.

For stimulation of chondrocytes, we used IL-1ß as the typical inductor of an inflammatory metabolism (Beekman et al. 1998). The important role of IL-1ß in the inflammatory process was shown by the analysis of synovial fluid samples from patients with rheumatoid arthritis and OA (Goldring et al. 1994). In our culture system, IL-1ß induced a distinct response of chondrocytes obtained from OA patients. Gene expression of MMP-3 was upregulated 44% and AGG downregulated 16%.

The alginate culture system we used is well established and allows the maintenance of the phenotype of articular chondrocytes (Häuselmann et al. 1992). Previous studies also showed that the metabolism of chondrocytes in this culture system is stable and comparable to the metabolism in vivo (Häuselmann et al. 1996).

In conclusion, in our inflammatory model of osteoarthrotic chondrocytes, inhibition of catabolism and anabolism was induced by dexamethasone. Our results suggest that dexamethasone inhibits proteoglycan metabolism.

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