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Lidocaine Potentiates the Chondrotoxicity of Methylprednisolone

Venkat Seshadri, M.D., Christian H. Coyle, Ph.D., and Constance R. Chu, M.D.

Cartilage Restoration Laboratory, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, U.S.A

Abstract

Purpose—This study examined the viability of bovine articular chondrocytes after exposure to methylprednisolone, methylprednisolone with lidocaine, and methylprednisolone in a simulated inflammatory environment.

Methods—Bovine articular chondrocytes were suspended in alginate beads and cultured in Dulbecco's modified Eagle's medium/F-12 for 1 week before experimentation. Suspended chondrocytes were exposed to 0.9% saline solution (negative control), methylprednisolone (4, 8, and 16 mg/mL), methylprednisolone (8 mg/mL) with 1% lidocaine, or methylprednisolone (8 mg/mL) and saline solution in a simulated inflammatory environment (interleukin [IL] 1 β exposure, 10 ng/mL) for 15, 30, and 60 minutes. Flow cytometry was performed 1 day, 4 days, and 7 days after exposure by use of annexin V and propidium iodide to assess chondrocyte viability.

Results—Chondrocyte viability decreased from 84% in saline solution to 62%, 38%, and 2.4% 1 day after 60 minutes of exposure to 4, 8, and 16 mg/mL of methylprednisolone, respectively ($n = 7$, $P < .05$). Chondrotoxicity increased with increasing time of exposure to methylprednisolone and with increasing time after exposure. In IL-1 β -activated chondrocytes, viability decreased from 76% in saline solution to 2.9% after 60 minutes of methylprednisolone exposure (8 mg/mL) ($n = 4$, $P < .05$). The combination of 8 mg/mL of methylprednisolone and 1% lidocaine further reduced viability to 1.0% after 60 minutes ($n = 4$, $P < .05$).

Conclusions—These results show a dose- and time-dependent decrease in chondrocyte viability after exposure to clinically relevant doses of methylprednisolone. The combination of methylprednisolone and lidocaine was toxic, with virtually no cells surviving after treatment. In addition, methylprednisolone did not mitigate the inflammatory effects of IL-1 β ; rather, it further potentiated the chondrotoxicity.

Clinical Relevance—Intra-articular injections of corticosteroids and local anesthetics are widely used in clinical practice. This in vitro study provides information on the potential effects of these drugs on articular cartilage.

Address correspondence and reprint requests to Constance R. Chu, M.D., Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, 200 Lothrop St, BSTWR E1640, Pittsburgh, PA 15261, U.S.A. chucr@upmc.edu.

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Keywords

Articular cartilage; Chondrotoxicity; Local anesthetics; Corticosteroids; Lidocaine; Methylprednisolone

Clinical case reports of unexplained chondrolysis in young patients after administration of continuous-infusion pain pumps have been described in the literature.¹ Numerous studies have alluded to the deleterious effects of local anesthetics on articular chondrocytes.^{1–5} Chu et al.² showed that 0.5% bupivacaine causes greater than 99% chondrocyte cell death after just a 15-minute exposure. In a more recent study Chu et al.³ showed that bupivacaine chondrotoxicity was dose- and time-dependent in bovine and human chondrocytes in vitro. Karpie and Chu⁴ showed the cytotoxicity of lidocaine on bovine articular chondrocytes in vitro. Because local anesthetics are so commonly given with corticosteroids, the next logical step would be to examine the potential effects of steroids on articular cartilage.

There have been studies in the literature that document the negative effects of steroids on articular cartilage. Mankin and Conger⁶ showed a profound decrease in protein synthesis, measured through radiolabeled glycine uptake, after the intra-articular injection of hydrocortisone in rabbit knees. Robion et al.⁷ showed an increased release of degradation products of aggrecan after methylprednisolone injections into horse radiocarpal joints. Nakazawa et al.⁸ showed chondrocyte apoptosis in steroid-treated human articular cartilage grafted onto the backs of mice with severe combined immunodeficiency.

However, corticosteroids have been shown to clinically reduce symptoms of pain from both osteoarthritis and inflammatory arthritis, primarily by suppression of synovial inflammation. Makrygiannakis et al.⁹ showed a decrease in expression of T-cell lymphocytes and inflammatory mediators in synovial fibroblasts after intra-articular corticosteroid injection in vivo. Interleukin (IL) 1 β is one such inflammatory marker that has been implicated in cartilage degeneration and osteoarthritis, through up-regulation of matrix metalloproteinases and down-regulation of inhibitors of matrix metalloproteinases.¹⁰ Exposing articular cartilage to IL-1 β has been used for in vitro studies to understand the early pathogenesis of osteoarthritis, as well as to provide a model for “stressed” chondrocytes.^{11–13}

This study was therefore performed to achieve the following goals:

1. To study the in vitro effects of methylprednisolone, a commonly used steroid, on the viability of bovine articular chondrocytes.
2. To study the effects of methylprednisolone on the viability of IL-1 β -activated chondrocytes.
3. To study the effects of the combination of methylprednisolone and lidocaine, as used clinically, on viability and to determine whether there is a synergistic effect.

We hypothesize that methylprednisolone alone, or in conjunction with lidocaine, is toxic to bovine articular chondrocytes in vitro in a normal environment and a simulated inflammatory environment.

METHODS

Articular chondrocytes obtained from freshly slaughtered bovine knees were used for this study. All flow cytometry experiments were conducted with bovine chondrocytes encapsulated in 3-dimensional alginate beads. For time-lapse imaging, chondrocytes in monolayer culture were imaged in situ by use of confocal microscopy.

Alginate Bead Cultures

Bovine knees were dissected to expose the articular cartilage in a sterile fashion. Finely minced cartilage harvested from these knees underwent enzymatic digestion with pronase for 90 minutes at 37°C, followed by collagenase P for 12 hours at 37°C. Chondrocytes recovered from the digestion were then encapsulated in alginate beads at a density of 4×10^6 cells/mL.¹⁴ Beads were kept in a tissue culture incubator at 37°C/5% carbon dioxide in chondrocyte growth medium consisting of Dulbecco's modified Eagle's medium/medium F-12 (50:50; Invitrogen, Grand Island, NY), plus 10% fetal bovine serum (Invitrogen) and 1% penicillin/streptomycin (Invitrogen), for 1 week before exposure to our experimental agents. For methylprednisolone, we used an aqueous suspension containing methylprednisolone acetate that is sparingly soluble in water. To improve its solubility, polyethylene glycol (PEG), an organic solvent, is included in the suspension. Dulbecco's modified Eagle's medium has previously been shown not to adversely affect bovine chondrocyte viability.¹⁵

Treatment Groups

After 1 week in culture, alginate beads were segregated into groups: beads exposed to methylprednisolone (experiment 1), beads activated with 10 ng/mL of IL-1 β and exposed to methylprednisolone (experiment 2), and beads exposed to methylprednisolone and lidocaine (experiment 3).

Experiment 1—Beads set aside for all experiments were segregated into groups of 10 beads each. Experimental groups were immersed in methylprednisolone at 3 different concentrations (4, 8, and 16 mg/mL) for 15, 30, or 60 minutes. Negative control groups were immersed in 1 mL of 0.9% normal saline solution for 60 minutes, and positive control groups were immersed in 1 mL of mono-iodoacetic acid (MIA) (6 mg/mL) for 60 minutes. One group of beads was set aside to assess the toxicity of the vehicle used to dissolve methylprednisolone (PEG); beads were exposed to 8 mg/mL of PEG. Samples were then washed and returned to chondrocyte growth media. Cell viability was assayed at 1 day, 4 days, and 7 days by flow cytometry as described later. Each experiment was repeated between 4 and 6 times by use of freshly isolated preparations of bovine articular chondrocytes within 3-dimensional alginate bead cultures from multiple animals. Results are presented as the mean of the total number of trials performed.

Experiment 2—After 1 week of culture, beads were incubated with chondrocyte growth medium supplemented with 10 ng/mL of bovine IL-1 β (AbD Serotec, Kidlington, England). One day after treatment, the experimental groups were immersed in 1 mL of methylprednisolone (8 mg/mL) and exposed for 15, 30, or 60 minutes. The negative control

group was exposed to 0.9% normal saline solution for 60 minutes. Medium was changed every other day with a fresh addition of IL-1 β .

Experiment 3—The experimental groups were immersed in 1 mL of methylprednisolone combined with 1% preservative-free lidocaine (to yield a final concentration of 8 mg/mL of methylprednisolone), whereas the negative control groups were immersed in 0.9% normal saline solution. Viability was assayed as described in experiment 1.

Flow Cytometry

At designated time points, 10 beads from each treatment group were removed for labeling by use of a Vybrant Apoptosis Assay Kit No. 3 (Molecular Probes, Eugene, OR). Alginate beads were dissolved in sodium citrate for 10 minutes and were recovered by centrifugation. Cells were washed twice in 1 $\times$ phosphate-buffered saline solution and resuspended in 400 μ L of 1 $\times$ Annexin Binding Buffer (Molecular Probes). To 100 μ L of each suspension, 5 μ L of Alexa Fluor 488 annexin V (Molecular Probes) and 1 μ L of propidium iodide were added for staining. These stained samples were incubated at room temperature for 15 minutes, and then 400 μ L of 1 $\times$ Annexin Binding Buffer was added. Samples were analyzed by a FACSDiva flow cytometry machine (BD Biosciences, Franklin Lakes, NJ) to identify annexin V- and propidium iodide-positive cells. Live cells were not stained, apoptotic cells had annexin V staining, and necrotic cells were stained with both annexin V and propidium iodide. Percentages of live, necrotic, and apoptotic cells were calculated by use of the total number of cells analyzed as the denominator. Figure 1 shows an example of a flow cytometry scatter plot 1 day after 1 hour of exposure to 8 mg/mL of methylprednisolone with 1% lidocaine and 0.9% normal saline solution (with necrotic cells in quadrant 2, viable cells in quadrant 3, and apoptotic cells in quadrant 4). For statistical comparison between experimental and control groups, data were analyzed by 1-way analysis of variance followed by Bonferroni *t* test by use of GraphPad software (GraphPad Software, San Diego, CA), with significance at $P < .05$. These experiments were repeated between 4 and 6 times by use of chondrocytes obtained from different bovine samples.

Real-Time Chondrocyte Imaging

To provide a second model of chondrocyte viability, time-lapse chondrocyte imaging was performed. Bovine articular chondrocytes cultured in monolayer were labeled with 2- μ mol/L 5-chloromethylfluorescein diacetate (Molecular Probes) and 1.5- μ mol/L propidium iodide for 30 minutes and then rinsed in HEPES-buffered media for 30 minutes. The labeled chondrocytes were exposed to either Hank's buffered saline solution (control), 1% lidocaine, 8 mg/mL of methylprednisolone, or a combination of lidocaine (1%) and methylprednisolone (8 mg/mL), placed in a temperature-controlled live-cell imaging stage (Delta T; Bioptics, Butler, PA) maintained at 37°C. Red (tetramethylrhodamine isothiocyanate) and green (fluorescein isothiocyanate) fluorescent channel image sets (Media Cybernetics, Rockville, MD) were acquired every minute by use of automated confocal microscopy (Olympus, Center Valley, PA). The percentage of dead cells (red) was calculated for each image set with Image-Pro Software (Media Cybernetics) and plotted over time. For statistical comparison between groups, percent viability was analyzed by Student *t* test at each time point, with significance set at $P < .05$.

RESULTS

Effects of PEG on Chondrocyte Viability

When the articular chondrocytes were exposed to PEG, the viability of the chondrocytes was similar to the negative control (normal saline solution) at 1 day, 4 days, and 7 days after exposure for all measured exposure times (15, 30, and 60 minutes).

Dose-Dependent Effects of Methylprednisolone Versus Normal Saline Solution

Flow cytometry showed that at 1 day after exposure, methylprednisolone caused a reduction in chondrocyte viability in a dose- and time-dependent manner. At 4 mg/mL, a 60-minute exposure of methylprednisolone caused a reduction in viability, from 84.1% in normal saline solution to 62.2% ($P < .05$). At double the concentration (8 mg/mL), viability decreased to 37.6% after 60 minutes ($P < .05$). At the highest concentration (16 mg/mL), viability decreased precipitously to 2.4% after a 60-minute exposure ($P < .05$) (Fig 2). Viabilities after 15- and 30-minute exposures were also significantly reduced compared with the negative control for 8 mg/mL and 16 mg/mL of methylprednisolone but were not significant at the lowest dose (4 mg/mL). Table 1 shows the complete results of these assays on post-exposure day 1, and Tables 2 and 3 show results for days 4 and 7, respectively ($n = 7$, $P < .05$).

IL-1 β -Activated Chondrocytes

After 1 week of culture in regular feeding media, a subgroup of alginate beads was exposed continuously to IL-1 β at 10 ng/mL. In this environment the chondrocytes were exposed to normal saline solution, and viability was assayed at 1 day, 4 days, and 7 days. A reduction in viability was seen over this 7-day period, from 75.8% at day 1 to 69.3% at day 4 to 59.6% at day 7 (Fig 3). These were significant at all time points when compared with the negative control group (normal saline solution) that was treated with regular media ($n = 10$, $P < .05$).

Effects of Methylprednisolone on IL-1 β -Activated Chondrocytes

Methylprednisolone did not counteract the inflammatory effects of IL-1 β but, in fact, further reduced the viability of the chondrocytes. One day after exposure to methylprednisolone (8 mg/mL), the viability of the chondrocytes decreased to 32.3%, 14.2%, and 2.9% after 15, 30, and 60 minutes of exposure. These values were significant when compared with the negative control of saline solution in the IL-1 β milieu ($n = 5$, $P < .05$) (Fig 4).

Methylprednisolone further potentiated the toxicity of IL-1 β . The viability of chondrocytes after exposure to 30 and 60 minutes of methylprednisolone was significantly lower with IL-1 β treatment ($P < .05$) when compared with non-activated chondrocytes.

Effects of Methylprednisolone (8 mg/mL) Combined With 1% Lidocaine

The combination of methylprednisolone and lidocaine was synergistic. On post-exposure day 1, viability was reduced to 17.5%, 2.8%, and 1.0% after 15, 30, and 60 minutes, respectively (Fig 5). These values were similar to the viabilities obtained with our positive control, MIA. Chondrocyte exposure to MIA for 60 minutes reduced viability to 8.0% ($n = 5$, $P < .05$).

Chondrocyte Viability 4 and 7 Days After Exposure

Reduction in viability was similar for all experimental groups except methylprednisolone at the lowest concentration (4 mg/mL). It should be noted that the percentage of viable cells recorded at days 4 and 7 is the percentage of the remaining surviving cells, because necrotic cells can be lost during media exchange. An interesting difference was observed in the composition of nonviable cells, when we compared the day 1 groups and the combined day 4 plus day 7 groups. The apoptotic fraction significantly increased from post-exposure day 1 to post-exposure days 4 and 7, whereas the necrotic fraction decreased ($n = 7$, $P < .05$) (Fig 6).

Real-Time Chondrocyte Imaging

Chondrocytes in monolayer culture showed reduction in viability to 67.1%, 38.0%, and 6.6% after 15, 30, and 60 minutes of exposure of methylprednisolone (8 mg/mL), respectively, when compared with Hank's buffered saline solution ($99\% \pm 0.3\%$ viable after 60 minutes, $n = 4$, $P < .05$). The combination of methyl-prednisolone and lidocaine greatly accelerated cell death; only 1.2% of the cells were viable after 30 minutes ($n = 4$, $P < .05$) (Fig 7).

DISCUSSION

This study shows that methylprednisolone, a commonly used corticosteroid, has dose- and time-dependent cytotoxic effects on bovine articular chondrocytes in vitro. This effect is synergistically increased when used in conjunction with lidocaine. Virtually no chondrocytes survived in vitro after exposure to the combination of methylprednisolone and 1% lidocaine. Furthermore, when chondrocytes were activated with IL-1 β , methylprednisolone did not counteract the cytotoxic effects of IL-1 β but further potentiated cell death.

Corticosteroids have been used for decades to treat joint pain and inflammation, with efficacious clinical results. A meta-analysis performed by Godwin and Dawes¹⁶ showed clinically and statistically significant reductions in osteoarthritic knee pain 1 week after injection and that the beneficial effects lasted 3 to 4 weeks after injection of corticosteroids. Methylprednisolone, which is used commonly, is an aqueous suspension containing methylprednisolone acetate, which is sparingly soluble in water. To improve its solubility, PEG, an organic solvent, is added to the suspension. Of note, there was no difference in chondrocyte viability between the negative control of normal saline solution and PEG; therefore PEG does not appear to be toxic to chondrocytes. Methylprednisolone has potency similar to triamcinolone, another commonly used intra-articular corticosteroid, approximately 5 times greater than hydrocortisone. Our study selected methylprednisolone because it is commonly used at our institution. Further comparison of effects of methylprednisolone, triamcinolone, and various other injectable glucocorticoids on articular cartilage warrants additional investigation.

Mankin and Conger⁶ evaluated the intra-articular effects of corticosteroids in vivo in rabbit knees by studying the time-dependent uptake of radiolabeled glycine in response to a single intra-articular dose of hydrocortisone. They showed that hydrocortisone depressed the

uptake of glycine, used as a surrogate for messenger ribonucleic acid and protein synthesis. This study raised the question as to whether corticosteroids, which are used ubiquitously in clinical practice, could potentially be detrimental to articular cartilage. Our study shows that clinical doses of methylprednisolone, when exposed to articular chondrocytes in vitro, resulted in a dose- and time-dependent increase in both necrosis and apoptosis when compared with saline solution exposure. On post-exposure day 1, most of the nonviable chondrocytes were necrotic, similar to what has been observed after exposure to bupivacaine and lidocaine, but on post-exposure days 4 and 7, the proportion of apoptotic cells significantly increased. This would suggest that initial insult caused cell necrosis but injured or damaged the remaining viable chondrocytes to release apoptotic signals, resulting in progressive cell death.

Chu and colleagues²⁻⁴ have shown the cytotoxic effects of local anesthetics such as lidocaine and bupivacaine in vitro. Because corticosteroids are commonly used with local anesthetics, our study attempted to show the combined effect of lidocaine and methylprednisolone on chondrocyte viability. What was seen was near-complete cell death in response to even a 15-minute exposure of chondrocytes to the combination of methylprednisolone and lidocaine. It has been hypothesized that the cytotoxic effects of local anesthetics are due to the initial disruption of the cell membrane, followed by the disruption of the mitochondrial transmembrane potential, leading to apoptosis.¹⁷

IL-1 β is an influential cytokine that is produced by activated synoviocytes, mononuclear cells, and articular cartilage itself.¹⁸ It significantly upregulates matrix metalloproteinases, a set of compounds implicated in cartilage matrix degeneration. It is also implicated in the excess production of nitric oxide by osteoarthritic tissues.¹⁸⁻²⁰ As a strategy to counter the negative effects of IL-1 β on articular cartilage, IL-1 receptor antagonists have been targeted as potential chondroprotective modalities in the treatment of osteoarthritis.²¹ Our study did not show a positive effect of methylprednisolone on IL-1-activated chondrocytes. Instead, a dramatic reduction in viability was seen, suggesting that methylprednisolone does not effectively counteract the effects of IL-1 β on articular chondrocytes. These data support the theory that the positive clinical effects of cortisone injections are a result of the anti-inflammatory effects of synoviocytes and not chondrocytes.

Several other investigators have studied the effects of glucocorticoids on articular chondrocytes in vitro and in vivo. Papacrhistou et al.²² observed a loss of cell organelles and distortion of cell shape with intra-articular injection of betamethasone in rabbit knees. Nakazawa et al.⁸ discovered increased chondrocyte apoptosis in corticosteroid-treated human articular cartilage grafted onto the backs of mice with severe combined immunodeficiency. Celeste et al.²⁰ showed an increase in markers of cartilage matrix degradation and aggrecan turnover with repeated triamcinolone injection into equine radiocarpal joints. However, Pelletier et al.¹⁹ showed that the intra-articular injections of methylprednisolone acetate in dogs reduced stromelysin (metalloproteinase) synthesis, macroscopic osteoarthritic lesions, and histologic changes using the Mankin score. Pelletier et al.²³ also showed a decrease in IL-1 β and expression of oncogene protein synthesis with triamcinolone injections in dog knees. In these studies different endpoints were chosen, different models were used, and different glucocorticoids were used with varying results.

Our study represents a direct assessment of chondrocyte viability after defined exposure to these agents using quantitative flow cytometry. Further work with different corticosteroids in vivo may clarify the effects of this class of agents on articular chondrocyte viability.

It is difficult to predict the concentration of methylprednisolone being exposed to the chondrocytes in a human knee joint after a single injection, because of the variability in synovial joint fluid, the presence of an effusion, and the surgeon's preference regarding dose and dilution (usually with a local anesthetic), as well as other factors. Methylprednisolone is typically packaged in 20-, 40-, or 80-mg/mL vials. A range of concentrations was selected (4 to 16 mg/mL) that would simulate concentrations seen when injecting methylprednisolone in the clinic. Our study shows a dose-dependent decrease in chondrocyte viability with methylprednisolone at ranges commonly used clinically at our institution. Importantly, the most common agents used clinically to dilute corticosteroids are local anesthetics, and these have a synergistic cytotoxic effect on the chondrocytes in vitro.

Major advantages of this study protocol include control and consistency over experimental conditions and direct quantitative measurements of chondrocyte viability. Immediate encapsulation was chosen instead of encapsulation after monolayer culture to maintain chondrocytes as close to their native phenotype as possible. Alginate is a natural hydrogel that allows for a more uniform distribution of chondrocytes than does articular cartilage and permits recovery of cells from suspension culture for flow cytometry. The advantage of using bovine articular chondrocytes was that we were able to collect large quantities of healthy tissue from multiple animals of similar age, breed, and sex. Chondrocyte activation with IL-1 β was used to provide an inflammatory stimulus to the articular chondrocytes to examine the effects of methylprednisolone on inflamed chondrocytes.

Limitations of this study include that it was an in vitro study of bovine chondrocyte viability within alginate beads. Although this model suspends chondrocytes in 3-dimensional culture, it does not precisely model in vivo conditions of chondrocytes within articular cartilage bathed in synovial fluid, and it does not study the effects on human chondrocytes. The human joint is a complex system involving the interaction among chondrocytes, synoviocytes, and synovial fluid. Therefore these results cannot be directly extrapolated to the clinical setting. However, the study does provide information on the toxicity of these agents to articular chondrocytes important to understanding potential effects of intra-articular administration.

This study clearly shows a dose- and time-dependent decrease in chondrocyte viability after exposure to methylprednisolone treatment and a synergistic decrease in chondrocyte survival with exposure to methylprednisolone combined with lidocaine in vitro. The critical finding from this study is that the negative effects observed on chondrocyte viability are dose and time dependent. Typically, surgeons inject 5- to 10-mL single injections of cortisone into human knees, generally spaced at least 3 months apart. To date, this has not resulted in clinical concern over cartilage damage, likely because this method of administration does not result in high enough agent concentrations for long enough periods of time to cause significant cell death and injury. This study further shows that steroids are not completely benign to articular chondrocytes and that there is potential for chondrotoxicity, especially

when 2 potentially toxic agents are used in combination. As such, the data support using the lowest concentration of steroids for the shortest period of time possible to achieve desired clinical goals.

CONCLUSIONS

These results show a dose- and time-dependent decrease in chondrocyte viability after exposure to clinically relevant doses of methylprednisolone. The combination of methylprednisolone and lidocaine was toxic, with virtually no cells surviving after treatment. In addition, methylprednisolone did not mitigate the inflammatory effects of IL-1 β ; rather, it further potentiated the chondrotoxicity.

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Figure 1.

Scatter plots 1 day after 60-minute exposures to 0.9% normal saline solution (A) and to methylprednisolone (8 mg/mL) combined with 1% lidocaine (B) show a significant decrease in cell viability ($P < .05$). Propidium iodide fluorescence (ordinate) is plotted against annexin V fluorescence (abscissa). Quadrant 2 (Q2) depicts necrotic cells, quadrant 3 (Q3) depicts live cells, and quadrant 4 (Q4) depicts apoptotic cells.

Figure 2.

Chondrocyte viability 1 day after exposure to 0.9% normal saline solution (60 minutes, negative control) versus 4, 8, and 16 mg/mL of methylprednisolone. Stars, $P < .05$ compared with normal saline solution.

Figure 3. Effects of IL-1 β (10 ng/mL) on chondrocyte viability with 1 day, 4 days, and 7 days of continuous exposure. Stars, $P < .05$ compared with normal saline solution.

Figure 4. Chondrocyte viability after exposure to methylprednisolone (8 mg/mL) in IL-1 β -stimulated environment with saline solution control (60 minutes). Stars, $P < .05$ compared with normal saline solution.

Figure 5.

Effect of methyl-prednisolone (8 mg/mL) alone, as well as the combination of methylprednisolone (8 mg/mL) and 1% lidocaine, on bovine articular chondrocyte viability, compared with saline solution control (60 minutes). Stars, $P < .05$ compared with normal saline solution.

Figure 6. Chondrocyte viability at day 1 and days 4 and 7 after exposure. An increase in the apoptotic fraction can be seen at the latter time points.

Figure 7.

Effects of methylprednisolone (8 mg/mL) and methylprednisolone with 1% lidocaine versus Hank's buffered saline solution (HBSS) on chondrocyte viability imaged in real time by use of live-cell confocal microscopy. The beads were continuously exposed to methylprednisolone and lidocaine and imaged in real time by use of live/dead staining. The cells were imaged for a total of 60 minutes, and at any given time, the percentage of viable cells is indicated. (scale bar, 180 μ m.)

Table 1

Percentage of Live Chondrocytes After Methylprednisolone, Methylprednisolone and Lidocaine, and IL-1 β Exposure Compared With Saline Solution 1 Day After Treatment

	15 min	30 min	60 min
Normal saline solution	—	—	84.1 $\pm$ 0.8
PEG (vehicle for methylprednisolone)	81.5 $\pm$ 4.6	83.1 $\pm$ 3.2	82.9 $\pm$ 3.5
Methylprednisolone, 4 mg/mL	74.6 $\pm$ 3.9	68.1 $\pm$ 6.0	62.2 $\pm$ 5.7 *
Methylprednisolone, 8 mg/mL	48.5 $\pm$ 5.6 *	46.0 $\pm$ 6.0 *	37.6 $\pm$ 9.8 *
Methylprednisolone, 16 mg/mL	7.4 $\pm$ 2.1 *	1.0 $\pm$ 0.3 *	2.4 $\pm$ 1.1 *
Methylprednisolone, 8 mg/mL, plus 1% lidocaine	17.5 $\pm$ 3.7 *	2.8 $\pm$ 1.0 *	1.0 $\pm$ 0.2 *
Normal saline solution in IL-1 β milieu	—	—	75.8 $\pm$ 2.0 *
Methylprednisolone, 8 mg/mL, in IL-1 β milieu	32.3 $\pm$ 9.2 *	14.2 $\pm$ 4.2 *	2.9 $\pm$ 1.6 *
MIA (positive control)	—	—	8.0 $\pm$ 12.3 *

* $P < .05$ versus saline solution control.

Table 2

Percentage of Live Chondrocytes After Methylprednisolone, Methylprednisolone and Lidocaine, and IL-1 β Exposure Compared With Saline Solution 4 Days After Treatment

	15 min	30 min	60 min
Normal saline solution	—	—	82.6 $\pm$ 1.3
PEG (vehicle for methylprednisolone)	83.4 $\pm$ 16.3	83.3 $\pm$ 6.3	81.8 $\pm$ 4.1
Methylprednisolone, 4 mg/mL	81.7 $\pm$ 1.7	77.2 $\pm$ 2.8	74.4 $\pm$ 4.0
Methylprednisolone, 8 mg/mL	57.0 $\pm$ 5.2 *	42.0 $\pm$ 3.4 *	20.6 $\pm$ 8.4 *
Methylprednisolone, 16 mg/mL	6.9 $\pm$ 2.6 *	0.7 $\pm$ 0.1 *	0.6 $\pm$ 0.2 *
Methylprednisolone, 8 mg/mL, plus 1% lidocaine	19.5 $\pm$ 9.0 *	2.6 $\pm$ 1.3 *	3.9 $\pm$ 2.9 *
Normal saline solution in IL-1 β milieu	—	—	69.3 $\pm$ 2.8 *
Methylprednisolone, 8 mg/mL, in IL-1 β milieu	35.8 $\pm$ 10.7 *	26.2 $\pm$ 1.9 *	1.1 $\pm$ 0.4 *
MIA (positive control)	—	—	3.7 $\pm$ 0.8 *

* $P < .05$ versus saline solution control.

Table 3

Percentage of Live Chondrocytes After Methylprednisolone, Methylprednisolone and Lidocaine, and IL-1 β Exposure Compared With Saline Solution 7 Days After Treatment

	15 min	30 min	60 min
Normal saline solution	—	—	84.3 $\pm$ 0.9
PEG (vehicle for methylprednisolone)	84.7 $\pm$ 2.6	84.8 $\pm$ 4.4	84.6 $\pm$ 2.2
Methylprednisolone, 4 mg/mL	78.0 $\pm$ 2.9	73.9 $\pm$ 4.7	74.8 $\pm$ 1.3
Methylprednisolone, 8 mg/mL	60.3 $\pm$ 6.6 *	57.5 $\pm$ 5.5 *	25.1 $\pm$ 11.9 *
Methylprednisolone, 16 mg/mL	3.6 $\pm$ 0.4 *	1.1 $\pm$ 0.5 *	0.8 $\pm$ 0.2 *
Methylprednisolone, 8 mg/mL, plus 1% lidocaine	4.9 $\pm$ 1.5 *	1.4 $\pm$ 0.1 *	1.4 $\pm$ 0.5 *
Normal saline solution in IL-1 β milieu	—	—	59.6 $\pm$ 2.0 *
Methylprednisolone, 8 mg/mL, in IL-1 β milieu	22.3 $\pm$ 6.1 *	15.4 $\pm$ 5.7 *	5.8 $\pm$ 3.5 *
MIA (positive control)	—	—	1.9 $\pm$ 1.6 *

* $P < .05$ versus saline solution control.