Knee Surg Sports Traumatol Arthrosc (2012) 20:1809–1814
DOI 10.1007/s00167-011-1820-6

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The chondrotoxicity of single-dose corticosteroids

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Received: 18 August 2011 / Accepted: 5 December 2011 / Published online: 21 December 2011

Abstract
Purpose Corticosteroids are commonly injected into the joint space. However, studies have not examined the chondrotoxicity of one-time injection doses. The purpose of this study is to evaluate the effect of dexamethasone sodium phosphate (Decadron®), methylprednisolone acetate (Depo-Medrol®), betamethasone sodium phosphate and betamethasone acetate (Celestone® Soluspan®), and triamcinolone acetonide (Kenalog®) on human chondrocyte viability in vitro.
Methods Single-injection doses of each of the corticosteroids were separately delivered to human chondrocytes for their respective average duration of action and compared to controls using a bioreactor containing a continuous infusion pump constructed to mimic joint fluid metabolism. A 14-day time-controlled trial was also performed. A live/dead reduced biohazard viability/cytotoxicity assay was used to quantify chondrocyte viability.
Results Over their average duration of action, betamethasone sodium phosphate/acetate solution and triamcinolone acetonide caused significant decreases in chondrocyte viability compared to control media ($19.8 \pm 2.9\%$ vs. $5.2 \pm 2.1\%$, $P = 0.0025$ and $10.2 \pm 1.3\%$ vs. $4.8 \pm 0.9\%$, $P = 0.0049$, respectively). In the 14-day trial, only betamethasone sodium phosphate/acetate solution caused a significant decrease in chondrocyte viability compared to control media (21.5% vs. 4.6%, $P < 0.001$).
Conclusions A single-injection dose of betamethasone sodium phosphate and betamethasone acetate solution illustrated consistent and significant chondrotoxicity using a physiologically relevant in vitro model and should be used with caution. Given the observed chondrotoxicity of triamcinolone acetonide in a single trial, there may be some evidence that this medication is chondrotoxic. However, at 14 days, betamethasone sodium phosphate and betamethasone acetate was the only condition that caused significant cell death.

Keywords Chondrocyte · Corticosteroid · Single-dose · Chondrotoxicity · Chondrolysis · Intra-articular injection

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Introduction

Osteoarthritis is one of the most common chronic debilitating diseases [22]. Corticosteroid injections are routinely used as an initial treatment option because they provide short-term symptomatic relief, reduce inflammation, and increase mobility [2, 5, 11, 19, 23]. There are several different types of intra-articular depot corticosteroids, however, the four most commonly used corticosteroids are dexamethasone sodium phosphate (Decadron®), methylprednisolone acetate (Depo-Medrol®), betamethasone sodium phosphate and betamethasone acetate (Celestone Soluspan®), and triamcinolone acetonide (Kenalog®) [5]. Each corticosteroid has a different solubility, serum half-life, average duration of action, and chemical properties [5, 15]. Presently, there are no guidelines with regard to the choice of corticosteroid; the decision is usually based on the physician's prior experience, cost, and availability [5, 10].

Previous literature examining the effect of these corticosteroids on articular cartilage is inconclusive. Several

animal models [10, 12, 18, 25] have demonstrated conflicting results for the effects of intra-articular corticosteroid administration on cartilage viability and integrity, while in vitro studies [14, 24] have documented pronounced chondrotoxicity. Conversely, several clinical studies examining the radiographs of patients with osteoarthritis who received repeated corticosteroid injections found that there were no detectable changes in the joint space [1, 20].

Repeated intra-articular injections of corticosteroids may have deleterious effects on tendon and cartilage [15, 25]; however, the effect of a single-injection dose of corticosteroids on the viability of chondrocytes has not been carefully considered [9]. The purpose of this study is to evaluate the chondrotoxicity of single-injection doses of the four most commonly used corticosteroids: dexamethasone sodium phosphate, methylprednisolone acetate, betamethasone sodium phosphate and betamethasone acetate, and triamcinolone acetonide on human chondrocytes over their average duration of action in order to assist the clinician when using an evidence-based approach to the use of intra-articular corticosteroid medications.

Materials and methods

Chondrocyte isolation and culture

Human, non-transduced, unmodified chondrocytes were obtained via a commercially available normal human chondrocyte line (Clonetics-Poietics, Walkersville, MD). Cultures were maintained at 37°C in humidified air containing 5% carbon dioxide during all experiments. Chondrocytes were then seeded at a density of 0.5×10^6 cells/well in six-well plates. Cells were grown in Dulbecco's modified Eagle medium (DMEM)/F-12 medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic antimycotic.

Bioreactor design

A bioreactor was created using a syringe pump (New Era Pump Systems, Inc., Model NE-1600-U) in order to simulate normal joint fluid metabolism. Syringes were connected to six-well plates using a 0.062-inch-diameter plastic tubing and O-rings. Four different corticosteroids were tested: dexamethasone sodium phosphate (Decadron®), methylprednisolone acetate (Depo-Medrol®), betamethasone sodium phosphate and betamethasone acetate (Celestone Soluspan®), and triamcinolone acetonide (Kenalog®). Media containing each corticosteroid, DMEM/F-12, 10% FBS, and 1% antibiotic antimycotic were delivered continuously to separate wells of the culture system.

The dosage of each corticosteroid was based on the dose equivalent of 40.0 mg triamcinolone acetonide, which is an accepted clinical dose. Dose adjustments were necessary to account for the scale difference of in vitro versus in vivo environments and could either be made according to absolute cell count or surface area measurements. The chondrocytes in this experiment were seeded at a density of 0.5×10^6 cells/well. The average number of chondrocytes present in human articular cartilage is 1.4×10^4 cells/mm^3, and average cartilage thickness ranges from 2 to 4 mm, resulting in an average density of 2.56×10^8 chondrocytes per knee [27]. This figure is 512 times greater than the number of cells in our study, but these values assume cartilage thickness greater than a single layer. Since our study consisted of only monolayer cells, corrections were instead based on surface area, which were believed to be more relevant. The average surface area of the human knee has been shown to be approximately 9,157 mm^2 [8], and the surface area of each well of a six-well plate is 960 mm^2. Accordingly, the dosages were decreased by a factor of eight to account for the reduction in cartilage surface area in this experiment. The dosages delivered to the chondrocytes can be referenced in Table 1. Each medication was respectively delivered to the chondrocytes over the average duration of action of each corticosteroid (Table 1) [5]. Each corticosteroid had its own respective duration dependent control (7-, 9-, and 14-day controls). Only DMEM/F-12, 10% FBS, and 1% antibiotic antimycotic were delivered to the controls. Each well of the plate was exposed to constant ingress/egress of the steroid and media mixture at a rate of 0.25 ml/h using a syringe pump (New Era Pump Systems, Inc., Model NE-1600-U). Egress occurred through drill holes on the sidewalls of the culture

Table 1 Solubility, average intra-articular residence time, and dosage equivalents for study medications

Generic name	Solubility (% wt/volume)	Average duration of action (days)	Dosage given (Equivalent dosage to 5.0 mg Kenalog)
Betamethasone sodium phosphate and betamethasone acetate (Celestone Soluspan®)	N/A	9	1.0 mg
Methylprednisolone acetate (Depo-Medrol®)	0.001	7	5.0 mg
Dexamethasone sodium phosphate (Decadron®)	N/A	7	1.17 mg
Triamcinolone acetonide (Kenalog®)	0.004	14	5.0 mg

plate. Each corticosteroid and control was tested in duplicate, and the trial was repeated.

An additional 14-day time-controlled trial was also performed to assess for time-dependent culture effects. Equivalent dosages and media controls were run over a 14-day period for all medications. Chondrotoxicity was evaluated after 14 days. Each corticosteroid and control was again tested in duplicate, and the trial was repeated.

Analysis

Live/dead viability/cytotoxicity assay (Invitrogen Corporation, Carlsbad, California), a two-color fluorescence assay, was used for the analysis of the chondrocyte cultures. Cellular viability was based on the simultaneous determination of live and dead cells with probes that recognize parameters of cell viability: plasma membrane integrity and intracellular esterase activity. Probes calcein acetoxymethyl ester (AM) and ethidium homodimer (EthD-1) were used. 20 µl of 2 mM EthD-1 was added to 10 ml Dulbecco's phosphate-buffered saline (D-PBS) while vortexing. The reagents were then combined with 5 µl of 4 mM calcein AM. This solution was then directly added to the chondrocytes. Cells were stained at room temperature for 30 min in the dark. The ratio of dead/live cells was assessed by fluorescent microscopy. Microscopic analysis was completed immediately after incubation using a Zeiss Observer Z1 (Carl Zeiss Inc, Thornwood, New York) fluorescent microscope. Images were taken using an AxioCam MRm camera (Carl Zeiss). Two frames were taken using the GP and DsRED2 filters, wavelengths 510 ± 10 and 590 ± 14, respectively, and were overlaid to produce a single image. Four random high-powered fields (100×) were acquired for each culture condition, totaling 16 HPF per condition. Each image was adjusted to maximize image quality and flat equalized. Automated cell-counting parameters were set based on the size of the smallest and largest cells visualized. Each color was then converted to a binary image, and stained cells that met threshold requirements were automatically counted. Preliminary studies determined that cells could be accurately identified if the number of contiguous pixels was $\leq$1,000 and $\geq$100. Manual counting was performed to validate automated cell-counting parameters. Manual and automated counts were equal to $\pm$4 cells. The ratio of dead/live cells was then calculated.

Statistical analysis

A mixed-effects regression model was used to compare percent cell death between each corticosteroid and its control media over its average duration of action. In the 14-day trial, a mixed-effects linear regression analysis was used to compare each corticosteroid to the control in terms of percent cell necrosis. Multiple comparisons were made using Tukey contrast. Results are reported as mean percent cell death $\pm$standard error.

Results

7-day trial

Dexamethasone sodium phosphate, which has an average intra-articular residence time of 7 days, did not produce a significant decrease in chondrocyte viability compared with 7-day media control ($9.2 \pm 1.5\%$ vs. $6.5 \pm 1.1\%$, n.s.) (Fig. 1). Similarly, methylprednisolone acetate did not produce a significant decrease in chondrocyte viability compared to the 7-day media control ($9.4 \pm 1.5\%$ vs. $6.5 \pm 1.1\%$, n.s.).

9-day trial

Betamethasone sodium phosphate and betamethasone acetate analysis demonstrated a significant decrease in chondrocyte viability when compared with the 9-day media control ($19.8 \pm 2.9\%$ vs. $5.2 \pm 2.1\%$, $P = 0.0025$) (Fig. 1). Betamethasone sodium phosphate and betamethasone acetate exhibited the largest decrease in chondrocyte viability overall.

14-day trial

Chondrocytes treated with triamcinolone acetonide for 14 days produced a significant decrease in viability compared with the 14-day media control ($10.2 \pm 1.3\%$ vs. $4.8 \pm 0.9\%$, $P = 0.0049$) (Fig. 1).

14-day time-controlled trial

A separate 14-day trial was performed with all medications and a single control to account for viability effects of culture time. Compared to the control media ($4.6 \pm 2.4\%$ cell death), dexamethasone sodium phosphate produced 1.3% more necrosis ($4.7 \pm 3.4\%$, n.s.), methylprednisolone acetate produced 38.2% more necrosis ($6.4 \pm 3.4\%$, n.s.), triamcinolone acetonide produced 98.3% more necrosis (9.2 ± 3.4, n.s.), and betamethasone sodium phosphate and betamethasone acetate produced 464% more necrosis ($21.5 \pm 3.4\%$, $P = 0.002$) (Fig. 2). Betamethasone sodium phosphate and betamethasone acetate produced significantly more necrosis compared to the other three corticosteroids, which were not significantly different from each other.

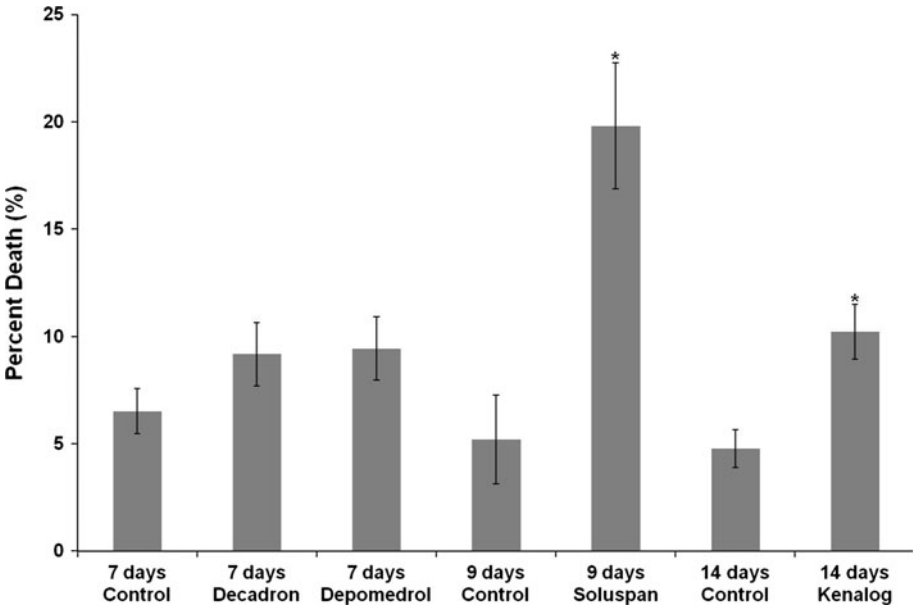

Fig. 1 The effect of each corticosteroid on cell viability after their respective duration of action. Data represent mean values ± standard error. $*P < 0.05$. Decadron® = Dexamethasone sodium phosphate, Depomedrol® = Methylprednisolone acetate; Soluspan® = Betamethasone sodium phosphate and betamethasone acetate (Soluspan Celestone®); Kenalog® = Triamcinolone acetonide

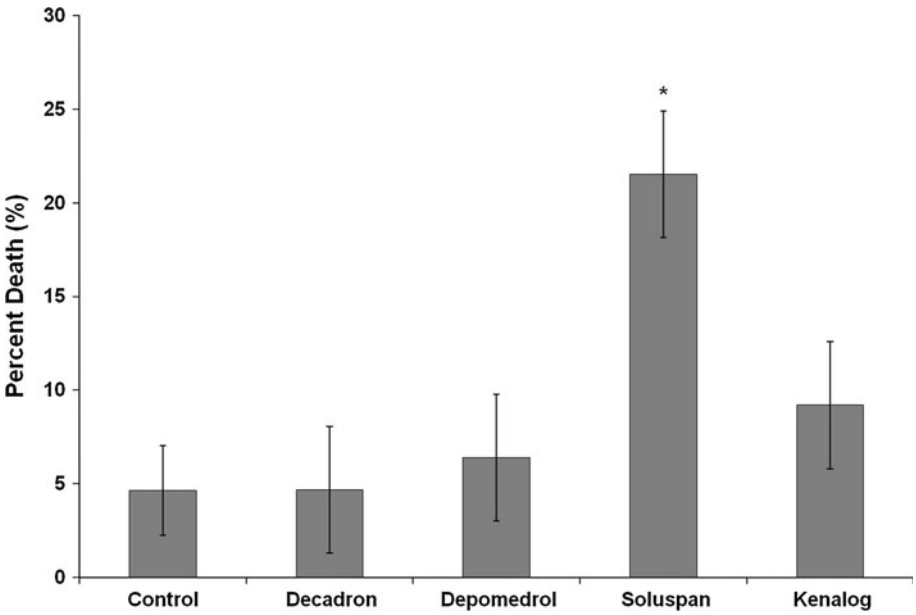

Fig. 2 The effect of each corticosteroid on cell viability after 14-day time-controlled trial. Data represent mean values ± standard error. $*P < 0.05$. Decadron® = Dexamethasone sodium phosphate, Depomedrol® = Methylprednisolone acetate; Soluspan® = Betamethasone sodium phosphate and betamethasone acetate (Soluspan Celestone®); Kenalog® = Triamcinolone acetonide

Discussion

The most important finding of the present analysis is that a single-dose concentration of betamethasone sodium phosphate and betamethasone acetate (Celestone Soluspan®) significantly decreased chondrocyte viability over its average duration of action compared with control cultures. Significant chondrotoxicity was also observed after assessing for time-dependent culture effects using a 14-day treatment period. Triamcinolone acetonide also demonstrated chondrotoxicity over its respective average duration of action only; however, betamethasone sodium phosphate and betamethasone acetate was the only corticosteroid to demonstrate significant chondrotoxicity in both trials.

Corticosteroid medications are often injected into the joint space to decrease inflammation. Even though there is evidence that these medications effectively reduce patient symptoms [2, 5, 18], little is known with regard to their potential chondrotoxicity. Although the exact mechanisms of chondrotoxicity of betamethasone sodium phosphate/acetate and triamcinolone acetonide are currently unknown, there are several plausible hypotheses. A recent in vitro study suggests that benzalkonium chloride, the preservative in the betamethasone sodium phosphate and betamethasone acetate solution, decreases chondrocyte viability [6]. Administered separately, neither betamethasone sodium phosphate nor betamethasone acetate induced chondrocyte death at individual concentrations twice as high as their concentration in betamethasone sodium phosphate/acetate solution. However, benzalkonium chloride significantly decreased chondrocyte viability starting at a dose of 10 μg/ml, which is one-twentieth of its concentration in betamethasone sodium phosphate/acetate preparation. At its concentration in betamethasone sodium phosphate/acetate, 200 μg/ml, benzalkonium chloride killed more than 99% of chondrocytes [6]. It has been hypothesized that benzalkonium chloride decreases cell viability by disrupting intermolecular interactions, causing dissociation of cellular membrane bilayers [6]. Methylprednisolone acetate, dexamethasone sodium phosphate, and triamcinolone acetonide do not contain benzalkonium chloride, which may help explain betamethasone sodium phosphate/acetate's observed toxicity.

Another possible mechanism of toxicity concerns the structure of betamethasone sodium phosphate/acetate crystals, which are physically similar to the monosodium urate crystals found in gout. Both are negatively birefringent [21] and have comparable sizes: 10–20 μm for betamethasone sodium acetate crystals and 1–20 μm for monosodium urate crystals [5, 16]. The similar shape of betamethasone and monosodium urate crystals may injure chondrocytes, releasing interleukin-1, inducible nitric oxide synthetase, and matrix metalloproteinases, which leads to cartilage destruction [4]. The size similarity may lead to macrophage injury, resulting in subsequent inflammation and cartilage destruction, as is seen with monosodium urate crystals. Unlike betamethasone sodium acetate crystals, both methylprednisolone acetate and triamcinolone acetonide have a positive birefringence and have an increased tendency to agglutinate [5], properties rendering them less similar to monosodium urate crystals. Further research is necessary to determine whether the crystalline structure itself leads to chondrotoxicity.

Although chondrotoxicity was not observed in cultures treated with triamcinolone acetonide in the combined 14-day time trial, this medication exhibited chondrotoxicity when compared with its control in the duration of action trial. Several studies have shown detrimental effects of triamcinolone acetonide on chondrocyte viability [3, 14, 28]. A recent investigation by Syed et al. [28] examined the effect of a single dose of triamcinolone acetonide alone and in combination with bupivacaine on both monolayer cultures of human chondrocytes and articular cartilage plugs. The authors found triamcinolone to be significantly chondrotoxic in both cases when compared with controls [28]. While these results partially corroborate our findings, some in vivo studies have shown that triamcinolone acetonide may not have deleterious effects in human patients with osteoarthritis [20] and may actually contribute to histopathological improvements in a canine model of osteoarthritis [17]. The conflicting results observed in this study coupled with the divided scientific literature indicate that a cautious clinical approach may be best when evaluating indications for the use of triamcinolone acetonide for single-dose intra-articular injections.

While the in vitro environment cannot replicate in vivo conditions, clinicians should consider the findings of this study when evaluating which corticosteroids to administer intra-articularly. Chondrotoxicity was not observed in cultures treated with dexamethasone sodium phosphate or methylprednisolone acetate, suggesting these medications may pose less risk to chondrocyte viability. Triamcinolone acetonide may pose some risk of chondrotoxicity. Conversely, the overwhelming chondrotoxicity observed with combinations of betamethasone sodium phosphate and betamethasone acetate suggests that this medication may be detrimental to chondrocyte viability and should be used cautiously.

A limitation of this study is that monolayer chondrocytes were analyzed instead of intact cartilage specimens, which may afford some protection to the chondrocytes. Although a physiologic bioreactor model and DMEM/F-12 media supplemented with FBS and 1% antibiotic antimycotic were used in attempt to mimic the intra-articular environment, these conditions are not equivalent to the in vivo joint environment and metabolism. A further limitation to this study is the use of a constant concentration of drug in vitro as a model of the clinical injection of a single dose. The pharmacokinetics of small-molecule drugs and tracers injected intra-articularly result in multi-exponential decay over the time course of hours [7, 13, 26]. Despite these limitations, we feel that constant-concentration exposure of cells in monolayer in a bioreactor is a conservative model, which is likely to estimate the combined pharmacokinetic and pharmacodynamic effects of each drug.

Conclusion

A single-injection dose of betamethasone sodium phosphate and betamethasone acetate solution illustrated consistent and significant chondrotoxicity using a physiologically relevant in vitro model. This chondrotoxicity may be due to the preservative benzalkonium chloride or due to betamethasone's crystalline structure. Given the observed chondrotoxicity of triamcinolone acetonide in a single trial, there may be some evidence that this medication is chondrotoxic. However, at 14 days, betamethasone sodium phosphate and betamethasone acetate was the only medication that caused significant cell death. Intra-articular injections of betamethasone sodium phosphate and betamethasone acetate should be used with caution due to its consistent and significant chondrotoxic effect in this investigation.

Acknowledgments The authors thank Alex Sox-Harris, PhD, for his assistance with data analysis.

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