Intervertebral disc cell protection of triamcinolone against bupivacaine toxicity

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ABSTRACT

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Background context: Local anesthetics combined with corticosteroids are commonly used for management of back pain in interventional spinal procedures. Several recent studies suggest cytotoxicity of bupivacaine, whereas others report protective and cytotoxic effects of corticosteroids on chondrocytes and intervertebral disc cells. Considering the frequent use of these agents in spinal interventions, it is meaningful to know how they affect intervertebral disc cells.

Purpose: This study was conducted to assess the effects of bupivacaine and triamcinolone, both alone and in combination, on viability of intervertebral disc cells *in vitro*.

Methods: Nucleus pulposus cells were isolated from human disc specimens from patients undergoing surgery due to disc herniation or degenerative disc disease. They were grown in three-dimensional alginate beads for one week to

maintain their differentiated phenotypes and to allow for matrix formation before analysis. After one week of culture, the cells were exposed to bupivacaine (0.1%, 0.25%, 0.5%, and 1%) or bupivacaine (0.1%, 0.25%, 0.5%, and 1%) with 1mg triamcinolone for 1, 3, or 6 hours. Cell viability was measured using trypan blue exclusion assay and flow cytometry. Live-cell/dead-cell fluorescent imaging was assessed using confocal microscopy.

Results: Trypan blue exclusion assays demonstrated dose- and time-dependent cytotoxic effects of bupivacaine on human nucleus pulposus cells. Similar but reduced cytotoxicity was observed after exposure to the combination of bupivacaine and 1mg of triamcinolone. Flow cytometry showed a dose-dependent cytotoxic effect of bupivacaine on nucleus pulposus cells after three hours of exposure. The reduced cytotoxicity of bupivacaine combined with 1mg triamcinolone was also confirmed in flow cytometry. Confocal images showed that the increase in dead cells correlated with the concentration of bupivacaine. Nevertheless, fewer cells died after exposure to several different concentrations of bupivacaine combined with 1 mg triamcinolone than did after exposure to bupivacaine alone.

Conclusions: The combination of bupivacaine and triamcinolone induced dose- and time-dependent cytotoxicity on human intervertebral disc cells *in vitro*, but the cytotoxicity was much weaker than that of bupivacaine alone. This study shows a potential protective influence of triamcinolone on intervertebral disc cells.

Key words : Bupivacaine; Triamcinolone; Intervertebral disc; Nucleus pulposus cell; Cytotoxicity; Pain blockage

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I. INTRODUCTION

Low back pain is a major public health concern with great medical and economic impacts. Low back pain is one of the main reasons for visiting a doctor.[1] The lifetime prevalence of back pain is 54%-80% in general population, and the annual prevalence of chronic low back pain is reported as 15%- 45%, with a point prevalence of 30%.[2]

Among the various causes of chronic low back pain, degeneration of the intervertebral disc is postulated as a major cause.[3, 4] Treating discogenic low back pain continues to be a challenge. Treatment for symptomatic degenerative lumbar disc disease is usually limited to conservative care, consisting of medication and physical therapy or arthrodesis.[5, 6] In recent years, several minimally invasive interventional techniques such as facet joint blocks,

selective nerve root blocks, sacroiliac joint blocks, intradiscal steroid injections, epidural or caudal steroid injections, intradiscal electrothermal therapy (IDET) and intradiscal radiofrequency (RF) thermocoagulation have been developed to reduce the need for surgery and to improve the quality of life for patients who require systemic drugs.[7-11] Interest is increasing in the development of physiological treatment options that address the underlying causes of spinal degeneration and pain, and spinal injection therapy is an alternative that introduces a symptom modifier and/or repair stimulant directly into degenerated and painful areas of the spine. Because of its advantages of minimal invasion and simplicity, injection therapy is adopted by many patients who are unwilling to undergo surgery. This has resulted in a sharp increase in the use of local injection as a tool for diagnosing and treating spinal pain.[12]

Although complications are possible with any invasive procedure, reports on spinal injection therapies indicate that they are relatively safe. Although rare, complications include hemorrhage, hematoma formation, dural puncture, intraarterial or intravenous injection, spinal cord trauma, nerve root injury, vascular injury, chemical meningitis, steroid side effects, and infection such as discitis, subdural abscess, epidural and paravertebral abscess.[11, 13] Most reported complications are related not to the direct effects of injected agents on the elements of the spine but to the procedure techniques. The increase in interventional spinal procedures is creating growing concern about the potential toxicity of injectable drugs to intervertebral disc cells. However, the direct effects of commonly used agents for injection therapy have not been well studied. Only recently have *in vitro* studies focusing on the effects of injection therapy on chondrocytes and disc cells been performed.

Agents used for spinal injection therapy can be single agents or mixed forms

that include local anesthetics such as lidocaine, ropivacaine, and bupivacaine, nonsteroidal anti-inflammatory drugs (NSAIDs), sarapin, sodium hyaluronate, morphine, or corticosteroids.[14] Local anesthetics and corticosteroids are usually used for interventional spinal procedures, both alone and in combination. Bupivacaine is a commonly used local anesthetic in interventional spinal procedures, used for diagnostic procedures and treatment of spine-related pain because of its long duration of neural blockade with reduced motor effects and neurotoxicity compared to those of lidocaine.[15] Recently, bupivacaine has been routinely used in facet joint injections, selective nerve root blockade, chemonucleolysis, epidural injections, intraoperative pain control during spine surgery, postoperative pain control after spine surgery, postprocedural pain control in intradiscal electrothermal treatment or discography, and intradiscal injection to reduce low back pain.[16-19] Local anesthetics such as bupivacaine relieve pain by inhibiting sensitization of nerve endings[20, 21] and reducing proinflammatory cytokine production.[22-25] Steroids are another commonly used agent for interventional spinal procedures.[26-28] Their anti-inflammatory effects partially contribute to the treatment of pain associated with inflammation,[29] which is implicated as a primary pain source either from direct chemical irritation or secondary to an autoimmune response to the nucleus pulposus.[30] Therefore, the rationale for using intradiscal steroids is the suppression of inflammation within the disc, resulting in the alleviation of symptoms.[31] Commonly, local anesthetics have been injected in combination with steroids to treat disc-related pain.[32, 33]

Although bupivacaine is one of the most commonly used local anesthetics for injection therapy, negative effects have been reported with its use, especially regarding its toxicity. A number of *in vitro* studies have demonstrated a dose-

and time-dependent chondrotoxic effect of bupivacaine, especially at clinically applied concentrations (from 0.1% to 1%).[34-37] Recently, the effects of bupivacaine on intervertebral disc cell viability were investigated, and three studies have suggested that bupivacaine may be toxic to intervertebral disc cells. *In vitro* exposure to bupivacaine of rabbit and human disc cells grown in monolayers or on three-dimensional alginate beads resulted in dose- and time-dependent cytotoxicity.[38] Another study demonstrated that bupivacaine is cytotoxic to intervertebral disc cells at clinically relevant concentrations.[39] The third study reported a dose- and time-dependent cytotoxic effect of bupivacaine on mouse disc cells in an organotypic culture system that approximates the *in vivo* matrix architecture.[40] These studies measuring toxicity to disc cells were limited to a single application of bupivacaine. Because local anesthetics are commonly administered with corticosteroids, the next reasonable step would be to examine the potential combined effects of local anesthetics and corticosteroids. However, combinational effects of local anesthetics and corticosteroids on human intervertebral disc cells have not been well explored. A few recent *in vitro* studies have reported harmful effects of the combination of corticosteroids and local anesthetics on articular cartilage,[41, 42] but no reports have examined the effects on intervertebral disc cells.

Therefore, the goals of this study were (1) to confirm the *in vitro* cytotoxic effect of bupivacaine on the viability of human intervertebral disc cells; and (2) to investigate the effects of the combination of bupivacaine and corticosteroids on viability. Triamcinolone, a commonly used clinically nondeleterious corticosteroid for injection therapy,[43-45] was used for this study.

II. MATERIALS AND METHODS

1. Disc cell isolation and three-dimensional alginate bead culture

Intervertebral disc tissue was obtained from patients who underwent a discectomy for disc herniation or interbody fusion for degenerative disc disease according to protocols approved by the Institutional Review Board (IRB) of Gangnam Severance Hospital, Yonsei University College of Medicine (No. 3-2010-0007). For homogeneous samples, disc materials were acquired from the nucleus pulposus not the annulus. Nucleus pulposus cells were isolated, individually resuspended in three-dimensional alginate bead cultures and cultured for seven days to maintain their differentiated phenotypes and to allow for matrix formation before analysis.[46, 47]

Nucleus pulposus cells were isolated through enzymatic digestion and were encapsulated in alginate beads at 4×10^6 cells per mL, as described by Masuda et al.[47] Alginate beads from a single patient's nucleus pulposus cells were placed in individual wells of a six-well plate and were housed in a tissue culture incubator at 37°C, 5% carbon dioxide in Dulbecco's modified Eagle's medium/F-12 (Invitrogen, Grand Island, NY, USA) (1:1), supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin/streptomycin (Invitrogen) for one week before exposure to experimental agents. Tissue culture medium was changed every three days.

2. Bupivacaine exposure of nucleus pulposus cells

After one week of culture, cells grown in alginate beads were exposed to 1 mL of 0.1%, 0.25%, 0.5%, or 1% bupivacaine for 1, 3, or 6 hours or were left

untreated as controls. Beads were washed twice with Hank's balanced salt solution with 1% penicillin/streptomycin and reincubated in growth media. After three days, cell viability was measured using a trypan blue exclusion assay and was reported as an average of independent trials using isolated preparations of nucleus pulposus cells from each patient. To confirm the dose-dependent effects of bupivacaine, flow cytometry and immunostaining were conducted to measure the cell viability of the three-hour treatment group.

3. Bupivacaine and triamcinolone exposure of nucleus pulposus cells

After one week of culture, the experimental group was exposed to 0.1%, 0.25%, 0.5%, or 1% bupivacaine combined with 1 mg of triamcinolone for 1, 3, or 6 hours, and the control group was treated with 1 mg of triamcinolone only. After exposure to bupivacaine and triamcinolone, bead washing, reincubation, and viability assays were conducted as above.

4. Analysis of cell viability

After bupivacaine exposure, cells released from beads were collected via centrifugation at 2000 rpm for five minutes and were resuspended in growth medium. Cells from each group were stained with 0.4% trypan blue solution for five minutes at room temperature. Cell suspensions were transferred to a hemocytometer for cell counting, and numbers of stained and unstained cells were determined under light microscopy by three independent observers. The trypan blue exclusion assay is based on the principle that live cells are not stained by dye, while dead cells are unable to exclude the dye because of their compromised plasma membrane structures.

At designated times, beads of nucleus pulposus cells were labeled using the

Vybrant Apoptosis Assay Kit #3 (Molecular Probes). After release, cells were resuspended in 400 μl of 1X Annexin Binding Buffer. To 100 μl of each suspension, 5 μL of Alexa Flour 488 Annexin V and 1 μL of propidium iodide (PI) were added to stain the cells. Cells were incubated at room temperature for 15 minutes, and 400 μL of 1X Annexin Binding Buffer was added. Samples were analyzed using flow cytometry to identify Annexin V- and PI-positive cells. Live cells were not labeled with either stain, apoptotic cells were stained by Annexin V only, and necrotic cells were stained by both Annexin V and PI.

To confirm cell viability, nucleus pulposus cells cultured in beads were labeled with 5-chloromethylfluorescein diacetate (live cells, green) and PI (dead cells, red) for 30 minutes and then rinsed in phosphate-buffered saline for 30 minutes. Labeled cells were imaged using confocal microscopy (Olympus, Center Valley, PA).

5. Statistical analysis

For statistical comparisons between experimental and control groups, data were analyzed using one-way analysis of variance (ANOVA), followed by Scheffe post hoc tests. The level of significance was set at $p<.05$. Error bars represent the standard deviations.

III. RESULTS

1. Effects of bupivacaine on nucleus pulposus cell viability

To test the effects of bupivacaine on nucleus pulposus cell viability, cells grown in three-dimensional alginate beads were exposed to bupivacaine at different concentrations for various periods of time, and cell viability was measured using a trypan blue exclusion assay and flow cytometry.

Trypan blue exclusion assays demonstrated dose- and time-dependent cytotoxic effects of bupivacaine on human nucleus pulposus cells. For cytotoxicity, results from untreated cells were set to 100%. Values less than 100% represent cell death caused by bupivacaine. After three hours of exposure to 0.1% bupivacaine, cell viability decreased to 80.0% ($p<.05$). Cell viability decreased to 65.0% with 0.25% bupivacaine, to 53.3% with 0.5%, and to 43.3% with 1% after three hours of exposure ($p<.05$). Cell viability after exposure to higher concentrations of bupivacaine was significantly reduced at all time points compared to that at lower concentrations of bupivacaine, except for viability after one hour of exposure to 0.1% bupivacaine. Time-dependent cytotoxicity of bupivacaine on nucleus pulposus cell viability was also observed. Viability after exposure to 0.1%, 0.25%, 0.5%, or 1% bupivacaine was significantly reduced in a time-dependent manner compared with that of the control, except for viability after one hour of exposure to 0.1% bupivacaine (Fig. 1). The results of trypan blue exclusion assays are included in Table 1.

Table 1. Relative viability of nucleus pulposus cells[1] after exposure to treatment reagents

	1 hour (%)	3 hours (%)	6 hours (%)
Bupivacaine 0.1%	93.3	80.0*	70.0*
Bupivacaine 0.25%	81.7*	65.0*	55.0*
Bupivacaine 0.5%	73.3*	53.3*	43.3*
Bupivacaine 1%	63.3*	43.3*	33.3*
Bupivacaine 0.1% + 1mg triamcinolone	97.2	91.7*	85.8*
Bupivacaine 0.25% + 1mg triamcinolone	93.3*	83.3*	75.5*
Bupivacaine 0.5% + 1mg triamcinolone	89.2*	77.5*	67.5*
Bupivacaine 1% + 1mg triamcinolone	84.2*	72.5*	63.3*

[1] measured using the trypan blue exclusion assay and calculated relative to control conditions (100%)

*p <.05 versus controls

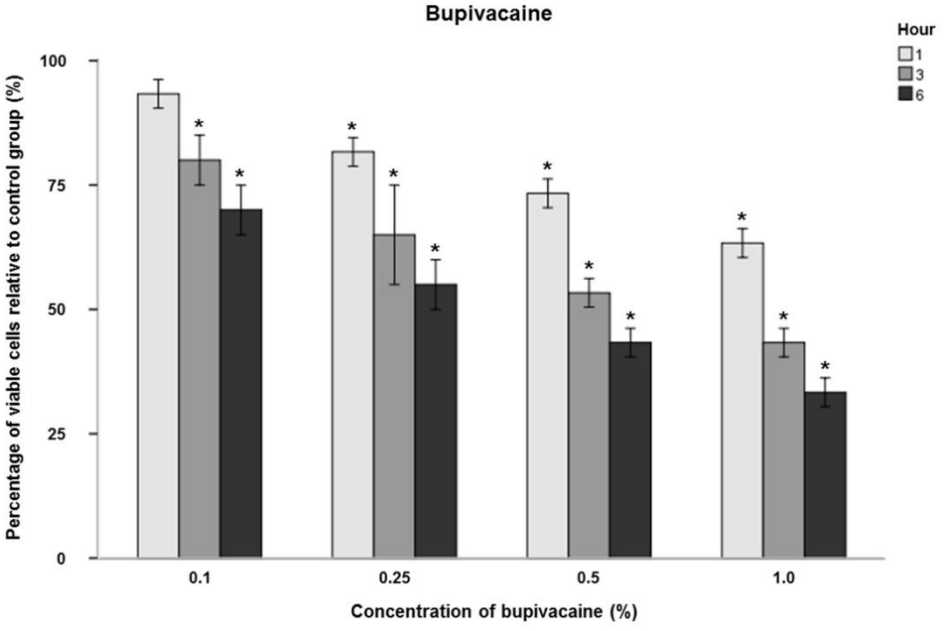

Fig. 1. Cytotoxicities of different concentrations of bupivacaine after 1, 3, or 6 hours of treatment, measured using the trypan blue exclusion assay and calculated relative to untreated control conditions (100%). Cell viability was significantly reduced in a dose- and time-dependent manner, except for viability after one hour of exposure to 0.1% bupivacaine. Data are mean ± standard deviation. *p <.05 compared with untreated controls.

Flow cytometry showed a dose-dependent cytotoxic effect of bupivacaine on nucleus pulposus cells after three hours of exposure. At 0.1%, a three-hour exposure to bupivacaine caused a reduction in viability from 95.8% in the no-treatment group to 84.1%. Cell viability decreased to 82.9% with 0.25% bupivacaine, to 64.8% with 0.5%, and to 59.1% with 1% after three hours of exposure (Fig. 2).

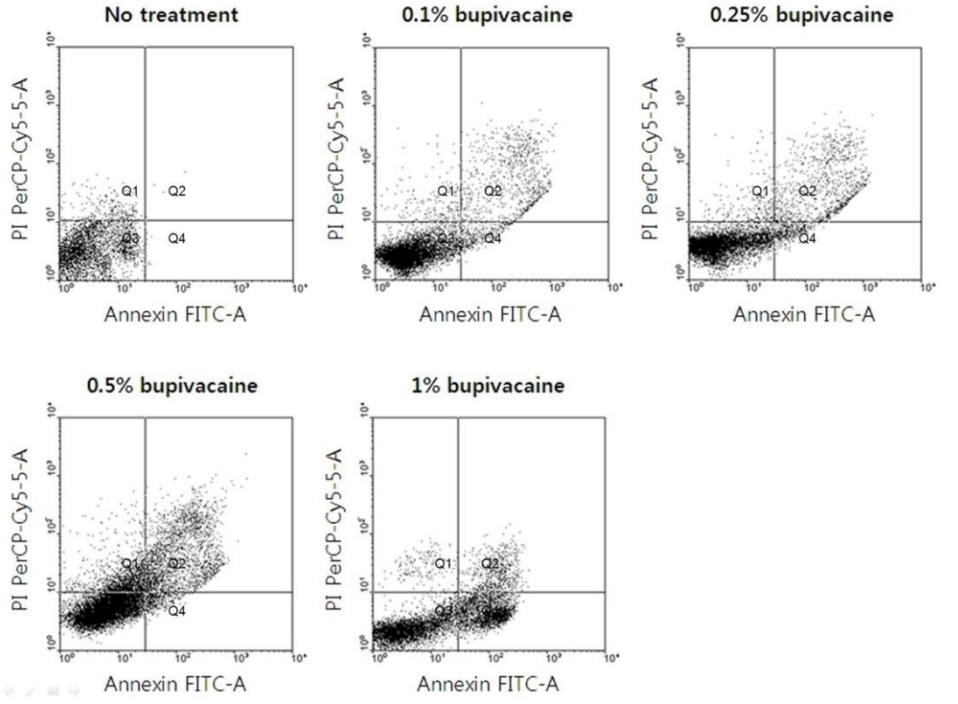

Fig. 2. Scatterplots of flow cytometry three days after three hours of exposure to different concentrations of bupivacaine. Quadrant 3 (Q3) shows live cells, quadrant 1 and 2 (Q1 and Q2) show necrotic cells, and quadrant 4 (Q4) shows apoptotic cells. Note the significant dose-dependent decrease in cell viability.

2. Effects of bupivacaine combined with 1 mg of triamcinolone

Bupivacaine combined with 1 mg of triamcinolone showed reduced cytoxicity on nucleus pulposus cells at every concentration. Cell viability was measured using a trypan blue exclusion assay, for which the cell viability of untreated control cells was set to 100%. Cell viabilities were reduced to 91.7%, 83.3%, 77.5%, and 72.5% after three hours of exposures to 0.1%, 0.25%, 0.5%, and 1% bupivacaine with 1 mg of triamcinolone, respectively ($p<.05$). Compared to the

viability after three hours of exposure to bupivacaine alone (80.0%, 65.0%, 53.3%, and 43.3% after exposure to 0.1%, 0.25%, 0.5%, and 1% bupivacaine), the cytotoxicity of the combination solution was much weaker. After one and six hours of exposure, the cytotoxicity of the combination on nucleus pulposus cells was also less than that of bupivacaine alone (Fig. 3). The results of trypan blue exclusion assays are included in Table 1.

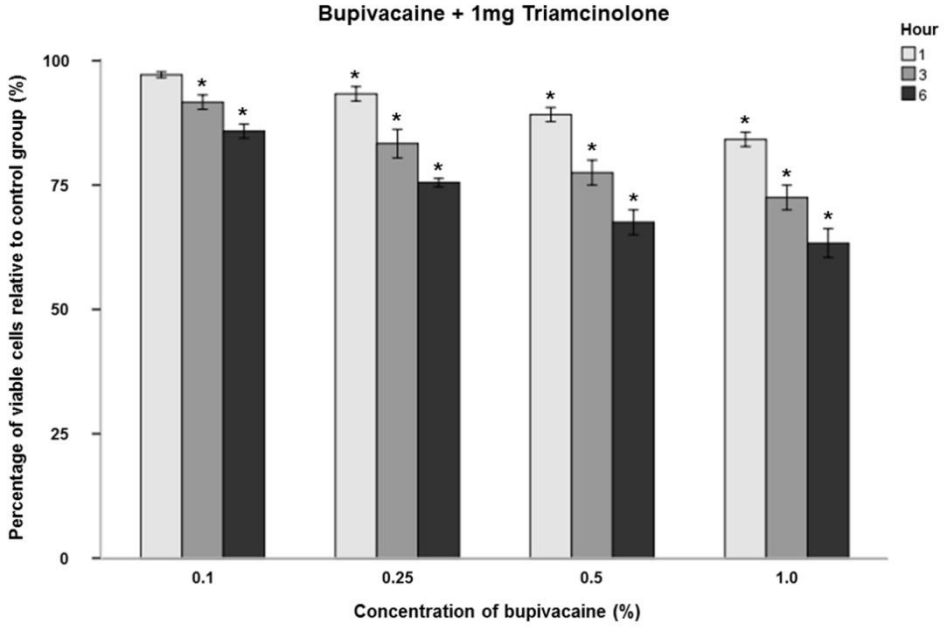

Fig. 3. Effects of different concentrations of bupivacaine and 1 mg triamcinolone on nucleus pulposus cell viability. Viability were measured using the trypan blue exclusion assay and calculated relative to control conditions (100%) treated with 1 mg triamcinolone. Dose-and time- dependent cytotoxicity of the combination on nucleus pulposus cells was observed, but the cytotoxicity was weaker than that of bupivacaine alone. Data are mean ± standard deviation. *p <.05 compared with controls treated with 1 mg triamcinolone.

Flow cytometry showed that the dose-dependent cytotoxic effect of the combination of bupivacaine and 1 mg of triamcinolone on nucleus pulposus cells after three hours of exposure was similar to that of bupivacaine alone. Nevertheless, the toxicity was much weaker than that of bupivacaine alone. Exposure to 0.1% bupivacaine and 1 mg of triamcinolone for three hours caused a viability of 95.0%, reduced from 98.7% in control cells treated with 1mg of triamcinolone alone. With 0.25%, 0.5%, and 1% combination solutions, cell viability decreased to 92.0%, 84.6%, and 76.8%, respectively, after three hours of exposure (Fig. 4).

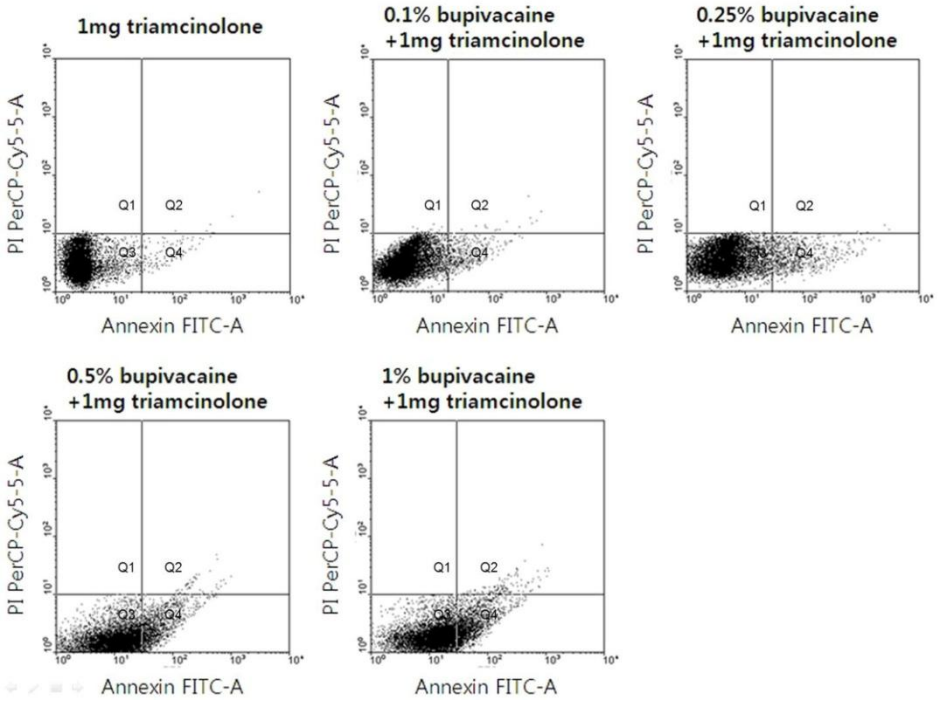

Fig. 4. Scatterplots of flow cytometry three days after three hours of exposure to bupivacaine and 1 mg triamcinolone. Quadrant 3 (Q3) shows live cells, quadrant 1 and 2 (Q1 and Q2) show necrotic cells, and quadrant 4 (Q4) shows apoptotic cells. Note the moderate dose-dependent decrease in cell viability. The number of dead cells was much less than that after exposure to bupivacaine alone.

3. Confocal microscopy

Viability staining with 5-chloromethylfluorescein diacetate (live cells, green) and PI (dead cells, red) under confocal microscopy showed a dose-dependent cytotoxicity of bupivacaine on nucleus pulposus cells after three hours of exposure. The confocal images showed that the increase in dead cells correlated

with the concentration of bupivacaine (Fig. 5, top). Analysis of nucleus pulposus cells treated with a combination of bupivacaine and 1 mg triamcinolone showed a protective effect of triamcinolone against the cytotoxic effects of bupivacaine. The confocal images showed that fewer cells died after exposure to several different concentrations of bupivacaine combined with 1 mg triamcinolone than did after exposure to bupivacaine alone (Fig. 5, bottom).

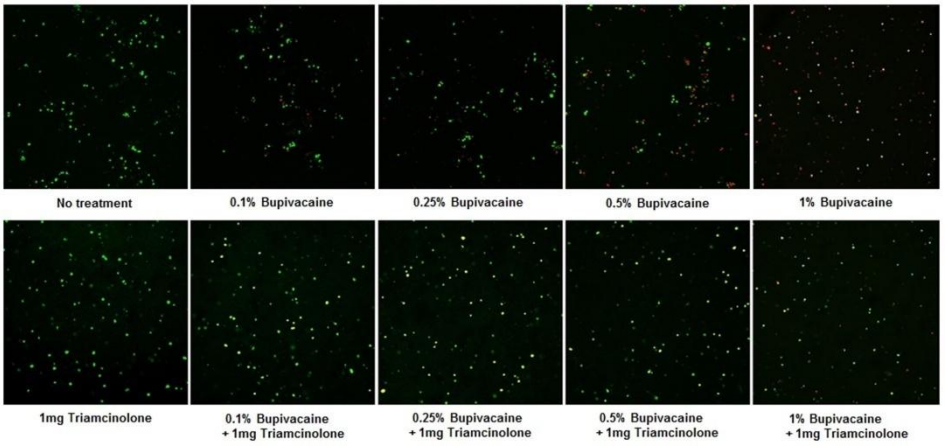

Fig. 5. Effects of different concentrations of bupivacaine and bupivacaine with 1 mg of triamcinolone on nucleus pulposus cell viability. Cell death was determined using 5-chloromethylfluorescein diacetate (live cells, green) and PI (dead cells, red) staining and imaging under confocal microscopy.

IV. DISCUSSION

Our results show that bupivacaine has dose- and time-dependent cytotoxic effects on human nucleus pulposus cells *in vitro*. This effects are decreased when used in combination with triamcinolone, a commonly used corticosteroid that showed a protective effect on nucleus pulposus cells against the cytotoxicity of bupivacaine in our study.

The observed cytotoxicity of bupivacaine to intervertebral disc cells is similar to that of recently published articles. Lee et al.[38] showed dose- and time-dependent cytotoxicity of bupivacaine to intervertebral disc cells grown in monolayer and alginate beads, mainly due to necrosis. Another study using an organotypic model system that mimics the *in vivo* environment showed that the cytotoxicity previously observed on isolated cells was also observed in excised mouse disc tissue.[40] Quero et al.[39] demonstrated that bupivacaine exhibited a cytotoxic effect on intervertebral disc cells at clinically relevant concentrations, although stimulation of cell proliferation occured at concentrations lower than 0.04%.

Whether the effects of steroids on intervertebral disc cells are toxic or protective is still under debate. Some studies have demonstrated that glucocorticosteroids stimulate chondrogenesis in chodrocytes.[48-51] Other studies have showed reduction of mechanically- and chemically-induced degeneration of articular cartilage after exposure to corticosteroids on a gross level.[52-54] Because the degeneration process is characterized by cell senescence and cell loss, a decrease in cell viability may increase the radiographic or histopathologic evidence. Therefore, a decrease in the degeneration of articular cartilage treated with corticosteroids implies protective effects of corticosteroids against cell death. Recently, a study showed a protective effect of dexamethasone on

intervertebral disc cells and viability induction of intervertebral disc cells under normoxic and hypoxic conditions.[55] Among readily available and clinically approved molecules, dexamethasone was regarded as a potential substitute for growth factors such as transforming growth factor beta (TGF-β), insulin-like growth factor I (IGF-I),[56] bone morphogenic proteins (BMP),[57] fibroblast growth factor-2 (FGF-2),[58] and growth differentiation factor-5 (GDF-5),[59] all of which are promising agents for the protection and regeneration of intervertebral disc. However, several other studies have showed cytotoxic effects of corticosteroids. A single dose of triamcinolone acetonide substantially increased chondrocyte apoptosis in human articular cartilage grafted onto the backs of mice with severe combined immunodeficiency.[60] Some studies have also found histologic evidence of chondrotoxicity from corticosteroids in experimental models.[61-63]

A few studies have reported the effects of corticosteroids combined with local anesthetics. Bolt et al.[64] showed that triamcinolone supported chondrocyte morphology in culture and protected chondrocytes from toxic effects exerted by mepivacaine on equine aricular cartilage. In contrast, a study using bovine cartilage found that the combination of lidocaine and methylprednisolone has a synergistic chondrotoxic effect.[41] In a study on human cartilage, the combined use of triamcinolone and bupivacaine caused a substantial loss of chondrocyte viability but did not demonstrate a synergistic chondrocidal effect.[42] We observed that triamcinolone exhibits protective effects on intervertebral disc cell viability. A decrease in the dose- and time-dependent cytotoxicity of bupivacaine was observed when bupivacaine was combined with triamcinolone. The differences between the studies may be multifactorial. Generalizations regarding chondrocytes used in studies of intervertebral disc cells may not be

possible. In addition, different corticosteroids and local anesthetics were used in the experiments, making it difficult to distinguish the individual effects of the drugs.

Limitations of our study include the following. First, our studies were performed *in vitro*, and the results may not reflect *in vivo* effects. Whether the same concentrations or durations of exposure of bupivacaine and triamcinolone would have the same effects *in vivo* in a clinical setting is unclear because of potential dilution, the extracellular matrix, and the avascular nature of the intervertebral disc. Considering the potential dilutional effects and wash out, disc cells *in vivo* would experience a lower concentration than those used in our experiment. A lower concentration of bupivacaine may not be sufficient to exhibit a cytotoxic effect on disc cells, but may exhibit a proliferative effect, as shown by Quero et al.[39] Although we chose immediate encapsulation in a three-dimensional alginate construct to maintain nucleus pulposus cells as close to their native state as possible, alginate is fairly homogenous and does not fully approximate the exquisite matrix structure of the disc. The next logical step would be an *in vivo* animal study; unfortunately, no clinical diagnostic tool exists to assess *in situ* disc cell death. Second, we chose to examine degenerative human disc cells, and whether the cells from degenerative disc tissues are more susceptible than cells from healthy disc tissues is not clear. However, studies on chondrocytes have indicated that an intact articular surface has a chondroprotective effects,[34, 42] suggesting that the natural native matrix structure may provide some protection, and that diseased tissue shows increased drug susceptibility. Third, our study used triamcinolone and bupivacaine because they are commonly used at our institution. Triamcinolone has a potency similar to that of methylprednisolone, another commonly used corticosteroid for

injection therapy, and which is approximately five times stronger than hydrocortisone. Bupivacaine is a common anesthetic used worldwide for pain control in interventional techniques. However, we recognize variability in drug choice and dosing among physicians. Therefore, further comparisons of effects of various corticosteroid and local anesthetics on disc cells remain to be investigated.

V. CONCLUSION

This study demonstrated dose- and time-dependent cytotoxic effects of bupivacaine on human intervertebral disc cells *in vitro*. The combination of bupivacaine and triamcinolone also exhibited dose- and time-dependent cytotoxicity, but the decrease in cell viability after exposure to the combination of drugs was much less than that observed after exposure to bupivacaine alone. These results show the potential of triamcinolone to have a protective influence on intervertebral disc cells recovering from various environmental stresses, including exposure to local anesthetics.

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ABSTRACT(IN KOREAN)

Bupivacaine toxicity에 대한 triamcinolone의 디스크 세포 보호 효과

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서론: 국소마취제와 코르티코스테로이드의 혼합제제는 요통의 치료를 위한 중재적인 척추 치료에 흔히 사용되고 있다. 최근 여러 연구에서 bupivacaine은 연골세포 및 디스크세포에 대해 독성을 갖는다고 밝혀지고 있으나, 이와는 다르게 코르티코스테로이드는 세포에 대해 독성을 나타내기도 하였고, 보호하는 효과를 보이기도 하였다. 최근 이 약물들을 사용하는 중재적 척추 치료가 증가함에 따라, 국소마취제와 코르티코스테로이드가 디스크세포에 어떤 영향을 미치는지 알아보는 것은 의미있는 연구가 될 것이다.

목적: 이 연구는 bupivacaine이 디스크세포에 미치는 독성을 확인하고, bupivacaine과 코르티코스테로이드의 혼합물이 디스크세포 생존력에 미치는 영향을 평가하고자 한다.

재료 및 방법: 수핵탈출증이나 퇴행성 디스크 질환으로 수술받은 환자로부터 인간 디스크 수핵세포를 추출한 뒤 디스크 세포의 특성을 잃지 않도록 삼차원 알지네이트 비드에서 일주일간 배양하였다.

일주일의 배양 후 세포들을 다양한 농도의 bupivacaine(0.1%, 0.25%, 0.5%, 1%) 또는 다양한 농도의 bupivacaine과 1mg의 triamcinolone의 혼합물에 1시간, 3시간, 6시간 동안 노출시켰다. 이후 3일간의 세포 배양 후 세포 생존은 트리판 블루 색소 배제법 및 유동세포계수법를 사용하여 측정되었다. 또한 면역 형광 염색후 공초점 현미경을 통해 세포의 생존을 확인하였다.

결과: bupivacaine의 용량 및 노출 시간이 증가할수록 인간 디스크 수핵세포에 대한 독성 효과가 강해짐을 트리판 블루 색소 배제법을 통해 확인하였다. 양상은 비슷하지만 감소된 독성이 bupivacaine 과 1mg의 triamcinolone의 혼합물에서도 관찰되었다. 3시간의 약물 노출 후 유세포 분석기를 사용한 평가에서도 약물 용량이 증가할수록 독성이 강해짐을 확인할 수 있었고, 혼합물에서 독성이 약해지는 결과를 보였다. 공초점 현미경을 통행 면역 형광 염색 이미지를 통해서 같은 결과를 확인할 수 있었다.

결론: 약물 용량 및 노출 시간에 비례하는 독성은 bupivacaine 단독으로 처리한 세포와 bupivacaine과 1mg의 triamcinolone의 혼합물로 처리한 세포 모두에서 나타났다. 하지만 bupivacaine 단독으로 처리한 세포에서 보다 혼합물로 처리한 세포에서 생존이 증가한다는 것을 관찰할 수 있었다. 이 결과를 통해 디스크 세포에 대한 triamcinolone의 잠재적인 보호 능력을 확인할 수 있었다.

핵심되는 말 : bupivacaine, triamcinolone, 추간판, 디스크, 수핵세포, 세포 독성, 통증 차단술