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Triamcinolone decreases bupivacaine toxicity to intervertebral disc cell in vitro

Ju-Hyung Moon, MD, Sung-Uk Kuh, MD, PhD*, Hyo-Suk Park, MS, Kyung-Hyun Kim, MD, Jeong-Yoon Park, MD, PhD, Dong-Kyu Chin, MD, PhD, Keun-Su Kim, MD, PhD, Yong-Eun Cho, MD, PhD

Department of Neurosurgery, Spine and Spinal Cord Institute, Gangnam Severance Spine Hospital, Yonsei University College of Medicine, 712 Eonjuro, Gangnam-gu, Seoul, South Korea

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Abstract

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.

STUDY DESIGN: Controlled laboratory study.

METHODS: Nucleus pulposus cells were isolated from human disc specimens from patients undergoing surgery because of disc herniation or degenerative disc disease. They were grown in three-dimensional alginate beads for 1 week to maintain their differentiated phenotypes and to allow for matrix formation before analysis. After 1 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 1 mg of 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 1 mg of triamcinolone. Flow cytometry showed a dose-dependent cytotoxic effect of bupivacaine on nucleus pulposus cells after 3 hours of exposure. The reduced cytotoxicity of bupivacaine combined with 1 mg of 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 of 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.

Keywords: Bupivacaine; Triamcinolone; Intervertebral disc; Nucleus pulposus cell; Cytotoxicity

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% to 80% in the general population, and the annual prevalence of chronic low back

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* Corresponding author. Department of Neurosurgery, Spine and Spinal Cord Institute, Gangnam Severance Spine Hospital, Yonsei University College of Medicine, 712 Eonjuro, Gangnam-gu, Seoul, South Korea. Tel.: (82) 2-2019-3404; fax: (82) 2-3461-9229.

E-mail address: kuhsu@yuhs.ac (S.-U. Kuh)

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pain is reported as 15% to 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 and intradiscal radiofrequency 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, intra-arterial or intravenous injection, spinal cord trauma, nerve root injury, vascular injury, chemical meningitis, steroid side effects, and infection such as discitis, subdural abscess, epidural abscess, and paravertebral abscess [11,13]. Most reported complications are related not only to the direct effects of injected agents on the elements of the spine but also 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, 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 with 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 spine-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 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 to confirm the in vitro cytotoxic effect of bupivacaine on the viability of human intervertebral disc cells and to investigate the effects of the combination of bupivacaine and corticosteroids on viability. Triamcinolone, a commonly used clinically