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Comparison of toxicity effects of ropivacaine, bupivacaine, and lidocaine on rabbit intervertebral disc cells in vitro

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Abstract

BACKGROUND CONTEXT: It has been shown that bupivacaine, the most commonly used local anesthetic to relieve or control pain in interventional spine procedures, is cytotoxic to intervertebral disc (IVD) cells in vitro. However, some other common local anesthetics, such as ropivacaine and lidocaine, are also frequently used in the treatment of spine-related pain, and the potential effects of these agents remain unclear.

PURPOSE: The purpose of this study was to evaluate the effect of various local anesthetics on rabbit IVD cells in vitro and further compare the cytotoxicity of ropivacaine, bupivacaine, lidocaine, and saline solution control.

STUDY DESIGN: Controlled laboratory study.

SUBJECTS: Rabbit annulus fibrosus (AF) and nucleus pulposus (NP) cells were isolated from Japanese white rabbits.

METHODS: Both AF and NP cells at the second generation maintained in monolayer were exposed to various concentrations of local anesthetics (eg, bupivacaine) or different durations of exposure and evaluated for cell viability by use of cell counting kit-8 (CCK-8). In addition, to compare the cytotoxicity of ropivacaine, bupivacaine, lidocaine, and saline solution control in commercial concentration, the viability was analyzed by flow cytometry after 60-minute exposure, and the morphologic changes were observed by the phase-contrast microscopy. Apoptosis and necrosis of IVD cells were confirmed by using fluorescence microscopy with double staining of Hoechst 33342 and propidium iodide.

RESULTS: Rabbit IVD cell death demonstrated a time and dose dependence in response to bupivacaine and lidocaine. However, ropivacaine only exerted a significant time-dependent effect on IVD cells. There was no significant difference in IVD viability after treatment with different doses of ropivacaine. In addition, the results showed that lidocaine was the most toxic of the three local anesthetics and that ropivacaine presented less cytotoxicity than lidocaine and bupivacaine. Fluorescence microscopy also confirmed that the short-term toxic effect of local anesthetics on both AF and NP cells was mainly caused by necrosis rather than apoptosis.

CONCLUSIONS: Results show that bupivacaine and lidocaine decrease cell viability in rabbit IVD cells in a dose- and time-dependent manner. All local anesthetics should be avoided if at all possible. Ropivacaine may be a choice if necessary, but it is also toxic. The increase in cell death is more related with cell necrosis rather than cell apoptosis. If these results can be corroborated in tissue explant models or animal studies, caution regarding diagnosing, treating, and controlling spine-related pain with local anesthetics is prompted. © 2014 Elsevier Inc. All rights reserved.

Keywords: Intervertebral disc; Bupivacaine; Lidocaine; Ropivacaine; Necrosis

FDA device/drug status: Not applicable.

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Introduction

Low back pain is the most common cause of limited activity in people younger than 45 years and the second most frequent reason for visits to the physician [1]. Because of its advantages of minimal invasion and simplicity, injection therapy is adopted by many patients who are unwilling to undergo surgery. Indeed, the use of local anesthetic injection as a tool in diagnosing and treating spinal pain has sharply increased from 1994 to 2001 [2].

Currently, bupivacaine is frequently used in interventional spine procedures and is often administered intraoperatively and postoperatively to control the pain by local, epidural, or spinal injection [3,4]. Extensive use of bupivacaine in a myriad of diagnostic procedures and pain management has been largely based on the assumption that it is safe. Recently, the effects of bupivacaine on intervertebral disc (IVD) cell viability were investigated, and four studies have suggested that bupivacaine may be toxic to IVD cells. Lee et al. [5] reported that bupivacaine could decrease viability of rabbit and human disc cells at a time and dose dependence. Similarly, application of bupivacaine in clinically relevant concentrations such as 0.25% or 0.5% was also toxic to human IVD cells [6]. Specifically, in vitro exposure of organotypic cultures of the murine functional spine units to bupivacaine solution also dramatically decreased cell viability and matrix protein synthesis in a dose- and time-dependent manner [7]. Moon et al. [8] recently confirmed that bupivacaine has dose- and time-dependent cytotoxic effects on human nucleus pulposus (NP) cells. Although aforementioned studies have shown cytotoxic effects of bupivacaine on IVD cells, it was still unclear whether this was specific to bupivacaine or could be seen with other local anesthetics. Furthermore, because both disc cell senescence and cell loss have been implicated in the development of IVD degeneration [9], an alternative, less cytotoxic local anesthetic could then be considered for diagnosis and treatment of low back pain.

A variety of other local anesthetics, such as ropivacaine and lidocaine, are also used, both alone and in combination, in diagnosing and treating spinal pain with little information regarding potential effects of these agents on IVD cells [10]. A promising alternative to bupivacaine for spine-related pain relief is ropivacaine. Ropivacaine is a long-acting aminoamide member of the pipecoloxylidide group of local anesthetics that differs from bupivacaine only by the replacement of the butyl group on the piperidine nitrogen atom of the molecule with a propyl group [11]. As a result, ropivacaine is less lipid-soluble than bupivacaine, and then together with its stereoselective properties, contributes to the property that has fewer cardiotoxicity and neurotoxicity than bupivacaine [12–14]. At the same time, lidocaine is another commonly used agent for interventional spinal procedures [15]. Lidocaine and bupivacaine are both members of the amide group in the local anesthetic family, but the action duration of lidocaine is approximately one-half that of bupivacaine [16]. It was shown that only 0.5% lidocaine has been effective in cervical epidural injections for managing chronic neck pain with disc herniation [17].

More importantly, recent studies also demonstrated that there are a number of detrimental effects of ropivacaine and lidocaine on cartilage and chondrocytes [18,19]. Lo et al. [20] observed that bupivacaine, ropivacaine, and lidocaine have a negative effect in a dose- and duration-dependent manner on chondrocyte viability. Park et al. [21] reported that bupivacaine and lidocaine have toxic effects on equine chondrocytes, and bupivacaine is the most toxic of the three local anesthetics in commercial concentration (ie, 2% lidocaine, 2% mepivacaine, and 0.5% bupivacaine). However, the results of Grishko et al. [22] suggested that 2% lidocaine may be the more toxic than 0.5% ropivacaine and 0.5% bupivacaine. In light of these observations, it would be prudent to examine the effects of three common local anesthetics on IVD cell viability, a subpopulation of which are chondrocytic in nature. It is also important to determine which local anesthetic may have the lowest cytotoxic effects on IVD cells, and then it could be a safer alternative drug than the others.

This study aimed to determine whether short-term exposures to bupivacaine, lidocaine, and ropivacaine decreased directly the viability of both rabbit annulus fibrosus (AF) and NP cells in a time- and dose-dependent manner in vitro and, furthermore, compare the toxic effects of three common local anesthetics with equipotent doses on rabbit IVD cells.

Materials and methods

As there is limited availability of normal nondegenerated human disc tissue, the normal and healthy IVD cells isolated from adult Japanese white rabbits were used in this study.

Isolation and culture of primary IVD cells

Experimental studies were conducted under the protocol approved by the animal experimentation committee of Huazhong University of Science and Technology. Annulus fibrosus and NP were surgically dissected from the thoracolumbar spine (L5–T10) of skeletally mature Japanese white rabbits (3 months, male) immediately after killing by air embolism. Cells were isolated as described previously [23,24]. Briefly, tissue from each level was collected, minced, and followed by enzymatic digestion. The resulting cells were seeded in six-well culture plates in Dulbecco's modified Eagle's medium/ham's F-12 (DMEM/F-12; Gibco, USA) containing 10% (for AF cells) or 20% (for NP cells) fetal bovine serum (Gibco, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco, Grand Island, NY, USA) at 37°C, 5% CO_2 atmosphere. Culture medium was changed every other day. After about

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