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Time- and dose-dependent cytotoxicities of ioxitalamate and indigocarmine in human nucleus pulposus cells

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Abstract

BACKGROUND CONTEXT: Ioxitalamate (Telebrix 300) is an ionic iodinated contrast medium commonly used for discography or percutaneous endoscopic lumbar discectomy (PELD), though it has side effects such as anaphylactic shock and renal toxicity. Indigocarmine is an organic compound dye with a distinctive blue color that is commonly used during PELD to stain the acidic, degenerated nucleus pulposus (NP). Although ioxitalamate and indigocarmine are widely used in spinal surgery, there have been no reports on their effects on NP cells. We studied the toxicities of both ioxitalamate and indigocarmine to NP cells.

PURPOSE: To determine the toxicities of both ioxitalamate and indigocarmine to NP cells in vitro.

STUDY DESIGN: In vitro, controlled study of the toxicities of both ioxitalamate and indigocarmine to human NP cells.

METHODS: Nucleus pulposus cells were obtained via discectomy from lumbar disc patients and isolated. Nucleus pulposus cells were cultured in three-dimensional (3D) alginate beads with 0.001, 0.1, 10, and 100 mg/mL ioxitalamate, 0.00001, 0.001, 0.1, and 10 mg/mL indigocarmine, or a mixture of both for 1, 2, or 3 days. The living cells were analyzed with trypan blue staining. Fluorescence Activated Cell Sorting analysis using Annexin V and propidium iodide and 3D alginate bead immunostaining was performed to identify live, apoptotic, and necrotic cells.

RESULTS: Ioxitalamate, indigocarmine, and their combination induced statistically significant NP cell injury that was both time- and dose dependent ($p<.05$). Also, at the same concentration, ioxitalamate was more cytotoxic than was indigocarmine or the combination ($p<.05$). All three treatments also showed dose-dependent cytotoxicity according to flow cytometry and immunostaining.

CONCLUSIONS: Ioxitalamate and indigocarmine are toxic to human NP cells in vitro in a time- and dose-dependent manner. We assume that ioxitalamate and indigocarmine may have similar effects in patients undergoing discography and PELD. Thus, we suggest that ioxitalamate and indigocarmine should be used carefully at low concentrations.

Keywords: Intervertebral disc; Contrast medium; Ioxitalamate; Indigocarmine; Cytotoxicity

FDA device/drug status: Not applicable.

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Introduction

The ionic iodinated contrast medium ioxitalamate (Telebrix 300) and indigocarmine are commonly used for diagnostic spinal procedures and spinal surgery. Ioxitalamate is often used in discography. Although magnetic resonance imaging (MRI) is a useful diagnostic tool for showing disc herniation, patients with degenerative disc disease are often asymptomatic, and physicians cannot directly assess disc herniations using MRI. Discography can allow physicians to determine if an abnormal disc is causing symptoms in these patients, although the validity remains controversial [1–6].

Indigocarmine enhances intradiscal visualization by preferentially staining the acidic, degenerated nucleus pulposus (NP). This staining helps the surgeon orient to the anatomy and selectively remove the herniated and unstable NP [7,8]. Also, during percutaneous endoscopic lumbar discectomy (PELD), indigocarmine is often mixed with ioxitalamate for confirmatory chromo-discography, an integral part of PELD. This combination produces radiopacity on discography, in addition to light blue chromatization of pathologic nuclei and annular fissures, which help to guide the targeted endoscopic fragmentectomy [9,10].

During the procedures, ioxitalamate and indigocarmine may directly affect NP cells when they are injected into the intradiscal space. Though ioxitalamate and indigocarmine are common in spinal surgery, there have been no reports on the effects of ioxitalamate and indigocarmine on NP cells. We, therefore, examined the toxicities of ioxitalamate, indigocarmine, and a mixture of both at various concentrations to NP cells in vitro.

Materials and methods

Isolation of human disc cells and three-dimensional alginate bead culture

Intervertebral disc materials were collected from 14 consented patients undergoing surgical procedures for lumbar spinal stenosis (n=8) and lumbar disc herniation (n=6). There were 9 men and 5 women with a mean age of 44.6 years (range: 23–67). The degree of intervertebral disc degeneration was evaluated using preoperative MRI according to the Pfirrmann grading system (Grade 3 n=5; Grade 4, n=9). The protocols were approved by the Institutional Review Board of Gangnam Severance Hospital, Yonsei University College of Medicine (No. 3-2010-0009). Because it is difficult to separate annulus fibrosus (AF) and NP tissues especially when disc materials were in high-graded degenerative status, we tried to obtain the disc material with en bloc fashion during operation, if possible. And we obtained NP from innermost area of en bloc disc material to ensure sample homogeneity, and the layer of the AF was dissected and discarded to avoid the contamination of cells. The tissue samples were first collected from the center portion of the NP. Nucleus pulposus cells were isolated through enzymatic digestion and were encapsulated in alginate beads at a density of 4×10^6 cells/mL, as described by Masuda et al. [11]. Five alginate beads made of patient's NP cells were placed in individual wells of six-well plates and incubated at 37°C, 5% carbon dioxide in Dulbecco modified Eagle medium (DMEM)/F-12 (Invitrogen, Grand Island, NY, USA) (1:1), supplemented with 360 μg/mL L-glutamine, 15 mM N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid, 10% fetal bovine serum (Invitrogen), and 1% penicillin/streptomycin (Invitrogen) for 1 week to maintain their differentiated phenotype and to allow the matrix to form before treatment [11,12]. The culture medium was changed every 3 days.

Treatment of NP cells

To examine the effects of contrast media, cells were treated with 0.001, 0.1, 10, and 100 mg/mL ioxitalamate, 0.00001, 0.001, 0.1, and 10 mg/mL indigocarmine, a combination of both, or DMEM (control) for 1, 2, or 3 days. The beads were then washed twice with Hank-balanced salt solution with 1% penicillin/streptomycin, and the growth media was replaced. Three days after treatment, cell viability was measured using a trypan blue exclusion assay and was reported as the average of three independent trials using NP cells from three different patients. On Day 2, NP cells from each treatment group were fluorescently labeled and analyzed using flow cytometry and immunofluorescence staining to identify apoptotic and dead cells.

Experimental solutions

Cells were incubated with either DMEM or various doses of ioxithalamate (Telebrix 300, 300 mg I/mL; Guerbet, Sulzbach, Germany), indigocarmine (Indigo Carmine Injection, 40 mg/mL; Phebra, Lane Cove, NSW, Australia), or the combination of the two (1:1 mass ratio) diluted in DMEM (ioxitalamate: 0.001, 0.1, 10, and 100 mg/mL; indigocarmine: 0.00001, 0.001, 0.1, and 10 mg/mL; and combination 0.00001, 0.001, 0.1, and 10 mg/mL). The experimental solutions are summarized in Table 1.

Determining cell viability using the trypan blue exclusion assay

After treatment, alginate beads containing the NP cells were dissolved by incubation for 20 minutes at 4°C in 5 volumes of a dissolving buffer (50 mM sodium citrate and 0.15 M sodium chloride [Fisher Scientific, Pittsburgh, PA, USA], pH 6.0). The resulting suspension was centrifuged at 4,000*g* for 5 minutes. Nucleus pulposus cells were resuspended in 1 mL of growth medium. Twenty microliters of cells was mixed with 20 μL of 0.4% trypan blue solution and incubated for 3 minutes at room temperature. Twenty microliters of that solution was transferred to a dual-chamber