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Basic Science

Which iodinated contrast media is the least cytotoxic to human disc cells?

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Abstract

BACKGROUND: Iodinated contrast media (CM) is commonly used for various intradiscal injections such as in discography and endoscopic spinal surgery. However, CM has been shown to be toxic to renal tissue due to its ionic strength and osmolarity and as a result of iodine-induced cytotoxicity, which has raised concern over whether there are similar negative effects on disc cells.

PURPOSE: This in vitro study was designed to identify the least cytotoxic iodinated CM to the human disc cell among four different physiochemical iodinated contrast dyes.

STUDY DESIGN: In vitro laboratory study.

METHODS: Intervertebral disc tissue was obtained by discectomy from a total of 10 lumbar disc patients undergoing surgery and disc cells were isolated. The human disc cells were grown in 3D alginate bead culture with 0, 0.1, 10, and 100 mg/mL CM solutions (ionic dimer, ionic monomer, non-ionic dimer, and non-ionic monomer) and mannitol as a control for 2 days. The living cells were analyzed with trypan blue staining. Fluorescence-activated cell sorting analysis was performed using Annexin V and propidium iodide (PI) and 3D alginate bead immunostaining to identify live, apoptotic, and necrotic cells.

RESULTS: Human disc cell death was time- and dose-dependent in response to CM and more necrosis was observed than apoptosis. In addition, non-ionic dimeric CM (iodixanol) showed the least toxic effect on human disc cells, followed by non-ionic monomeric (iopromide), ionic dimeric (ioxaglate), and ionic monomeric CM (ioxithalamate).

CONCLUSIONS: Contrast media is cytotoxic to human disc cells in a dose- and time-dependent manner. This in vitro study revealed that, among four different CM preparations, non-ionic dimeric CM is the least detrimental to human disc cell viability. Careful attention should be paid to the type of CM chosen for discography and endoscopic spinal surgery. It is also necessary to investigate the detrimental effects of CM on disc cells and disc degeneration in further in vivo studies.

Keywords: Contrast media; Osmolarity; Cytotoxicity; Disc; Ionic; Nonionic

Introduction

The increase in interventional spinal procedures is creating growing concern about the potential toxicity of injectable drugs to intervertebral disc (IVD) cells. However, the direct effects of agents commonly used for injection therapy have not been well studied. Among these injectable drugs, iodinated contrast medium is often used for diagnostic and therapeutic spinal procedures such as endoscopic lumbar discectomy, intradiscal electrothermal therapy, discography, facet joint block, chemonucleolysis, or selective nerve root blockade [1]. Intradiscal injection of local

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anesthetics such as bupivacaine and corticosteroids has been under investigation and several studies reported cytotoxic or proliferative effects [2–4].

However, to our knowledge, the response of IVD cells to contrast media (CM) is not well reported, although the cytotoxicity of CM to various renal cells has been thoroughly investigated [5–8]. There are also numerous comparative studies examining different nephrotoxic influences with respect to physiochemical properties such as ionicity (non-ionic vs ionic) and molecular structure (monomeric vs dimeric) [9–12]. There are four categories of CM: ionic monomeric, ionic dimeric, non-ionic monomeric, and non-ionic dimeric [13]. The exact mechanism of CM cytotoxicity remains unclear and debate continues due to varying experimental conditions. However, many studies have reported that non-ionic CM is less nephrotoxic than ionic CM based on in vivo and in vitro experiment and that dimeric CM has more desirable properties than monomeric CM with regard to cytotoxicity [14,15].

Thus, the current in vitro study was designed to determine the cytotoxic effect of CM on human disc cells according to concentration and physiochemical properties. We also aimed to identify the least cytotoxic CM by comparing ionic strength, osmolarity, molecular structure (monomeric or dimeric), and viscosity.

Materials and methods

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

Intervertebral disc materials were collected from 10 consenting patients undergoing surgical procedures for degenerative lumbar disease. There were three men and seven women with a mean age of 50.9 years (range, 23–70). The degree of IVD degeneration was evaluated using preoperative magnetic resonance imaging according to the Pfirrmann grading system (Grade 3: n=7, Grade 4: n=3). The protocols were approved by the Institutional Review Board of OOO Hospital, OOO University College of Medicine (the name of institute has been withheld) (No. 3-2012-0258).

Because it is difficult to separate annulus fibrosus and nucleus pulposus (NP) tissues, especially when disc materials are of high-grade degenerative status, we attempted to obtain the disc material in an en-bloc fashion during operation when possible. We obtained NP from the innermost area of the en-bloc disc material to ensure sample homogeneity, and the layer of the annulus fibrosus was dissected and discarded to avoid contamination of the cells. The tissue samples were first collected from the center portion of NP. Nucleus pulposus cells were isolated through enzymatic digestion and were encapsulated in five alginate beads at a density of $4x10^4$ cells/mL, as described by Masuda et al [16]. Five alginate beads made of patient NP cells were placed in individual wells of six-well plates and incubated at 37°C in 5% carbon dioxide in Dulbecco's modified Eagle's medium/F-12 (Invitrogen, Grand Island, NY) (1:1) supplemented with 360 μg/mL L-glutamine, 15 mM HEPES, 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 [16,17].

Treatment of NP cells

To examine the effects of CMs, cells were treated with 0, 0.1, 1, 10, and 100 mg/mL CM solutions (ionic monomer, ionic dimer, non-ionic monomer, and non-ionic dimer) or mannitol (control) for 2 days. The beads were then washed twice with Hank's balanced salt solution with 1% penicillin/streptomycin, and the growth media was replaced. Two 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.

Physicochemical properties of different CM

The detailed physiochemical properties of the experimental solutions are summarized in Table. We used four different CMs according to ionicity (ionic, nonionic) and number of benzene rings (monomeric, dimeric). Cells were incubated with either Dulbecco's Modified Eagle's medium or various doses of ionic monomer (ioxithalamate, Telebrix 300, Guerbet, Sulzbach, Germany), ionic dimer (ioxaglate, Hexabrix 320, Guerbet, Sulzbach, Germany), non-ionic monomer (iopromide, Ultravist, Schering, Berlin, Germany), or non-ionic dimer (iodixanol, Visipaque, GE Healthcare, AS Oslo, Norway).

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 five volumes of dissolving buffer comprising 50 mM sodium citrate (Fisher Scientific, Pittsburgh, PA) and 0.15 M

Table
Physicochemical properties of different contrast media

Contrast media	Property	Osmolarity at 20°C (mOsm/kg H2O)	Viscosity at 37°C (cp)	Iodine content (mg/mL)
Ioxithalamate	Ionic monomer	1,710	5.2	300
Ioxaglate	Ionic dimer	600	15.7	320
Iopromide	Nonionic monomer	590	8.9	300
Iodixanol	Nonionic dimer	290	26.6	320

sodium chloride (Fisher Scientific), pH 6.0. The resulting suspension was centrifuged at 4,000 g for 5 minutes. NP cells were resuspended in 1 mL of growth medium, and 20 μL of cells were mixed with 20 μL of 0.4% trypan blue solution and incubated for 3 minutes at room temperature. Twenty microliters of that solution were then transferred to a dual-chamber hemocytometer, and the cells were counted. The number of living cells was determined by three independent observers using light microscopy. The trypan blue does not stain living cells because their membranes remain intact.

Determining cell viability using flow cytometry and immunostaining

Cells from each treatment group were labeled using the TACS Annexin V Kit (Trevigen Instructions). After releasing the cells, they were resuspended in 1 mL of 1X Annexin Binding Buffer. One microliter of Alexa Flour 488 Annexin V, 10 μL of PI, 10 μL binding buffer, and 79 μL distilled water were added to stain the cells. Cells were incubated at room temperature for 15 minutes, then 1 mL of 1X Annexin Binding Buffer was added. Annexin V- and PI-positive cells were identified using flow cytometry. Live cells were not labeled by either stain, apoptotic cells were stained by Annexin V, and necrotic cells were stained by PI.

To confirm cell viability, live and dead cells were imaged using confocal microscopy. NP cells cultured in beads were labeled with 5-chloromethylfluorescein diacetate (live, green) and propidium iodide (dead, red) for at least 1 hour and then rinsed in phosphate-buffered saline for 30 minutes. The labeled cells were imaged using confocal microscopy (Olympus, Center Valley, PA).

Statistical analysis

The data are reported as living cell counts or percentages of living cells and are presented as the mean ± the standard deviation. Data were analyzed using two-way ANOVA followed by Tukey's post hoc test to compare the effect of the type of experimental solution and concentration on live cell counts. A p-value less than 0.05 was considered statistically significant.

Results

Dose-dependent effects of various CM on the viability of human NP cells

Cell viability according to the trypan blue exclusion assay

To assess dose-dependent toxicities of four CM preparations on NP cells, we counted living NP cells using the trypan blue exclusion assay. Living cells significantly decreased at increasing concentrations of CM (Fig. 1). As shown in Fig. 1, among the CMs, the ionic monomeric CM showed the greatest cytotoxic effect followed by the ionic dimeric, non-ionic monomeric, and non-ionic dimeric CMs (p-value <.005; Fig. 1).

Cell viability according to flow cytometry and confocal microscopy

As shown in Fig. 2, scatter plots generated from flow cytometry results for cells exposed to different concentrations of CM showed that there were more scattered dots in Quadrant 3 (representing live cells) than in Quadrants 1, 2, or 4 (representing necrotic or apoptotic cells) for the lower concentration and non-ionic dimeric CM (Fig. 2). As the

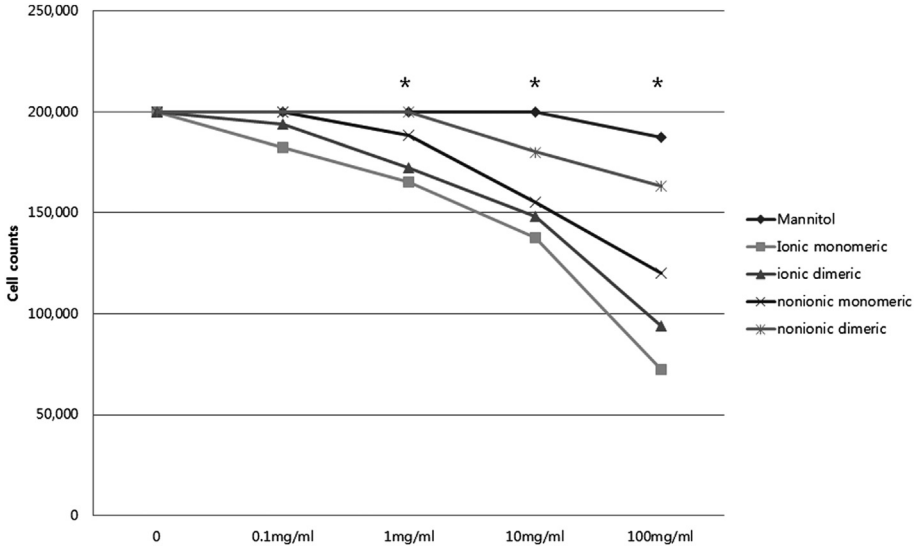

Fig. 1. A comparison of cytotoxicities of different concentrations of various contrast media, measured using the trypan blue exclusion assay. The least cytotoxicity was found by nonionic dimeric contrast media, and ionic monomeric contrast media showed the greatest cytotoxic effect. *p-value <.05, 2-way ANOVA with post hoc tukey's test.

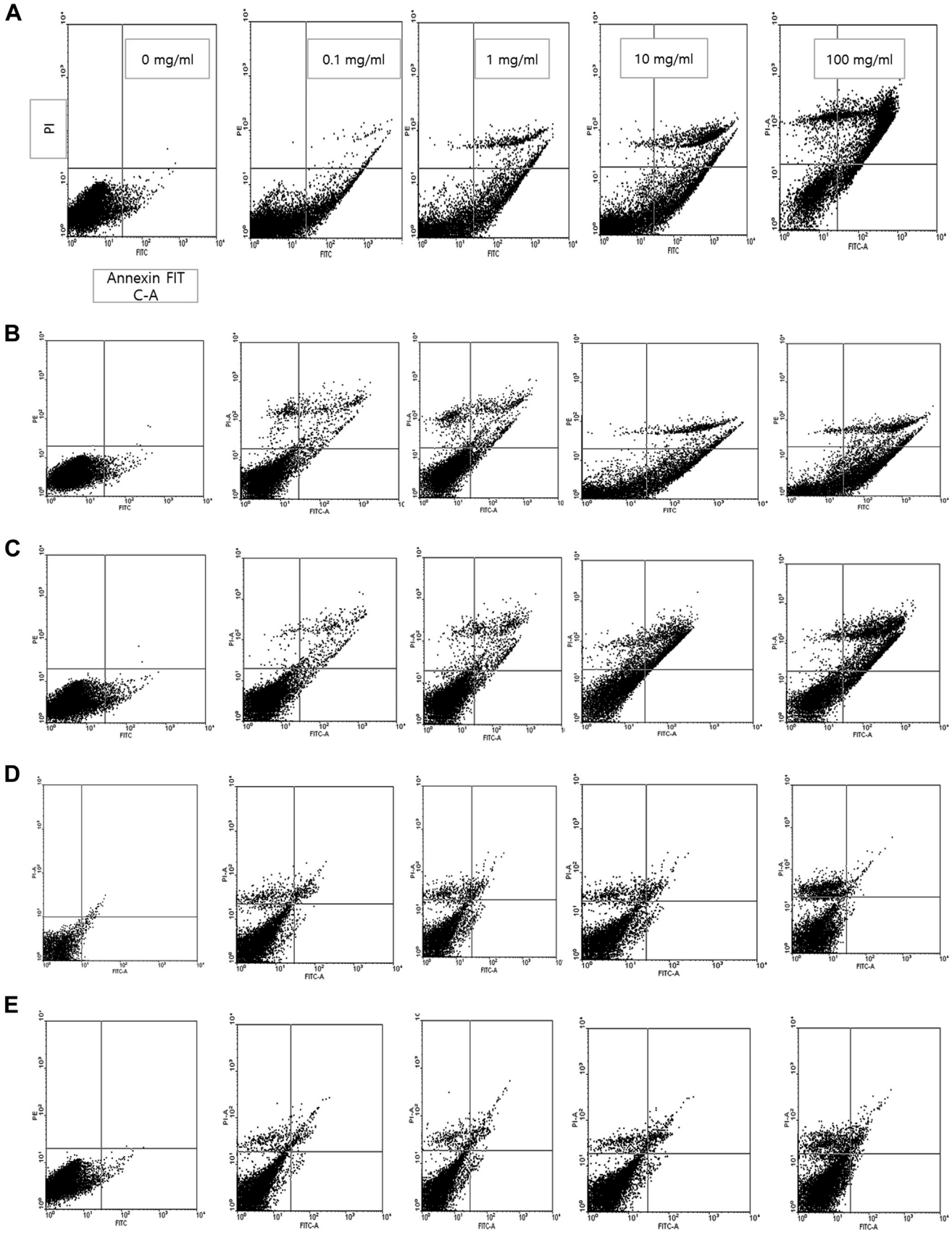

Fig. 2. The results of scatter plots of flow cytometry exposure to different concentrations of various contrast media. Quadrant 3 (Q 3) shows live cells, Quadrants 1 and 2 (Q1 and Q2) show necrotic cells, and Quadrant 4 (Q4) shows apoptotic cells. Note the least toxicity was shown in nonionic dimeric contrast media on cell viability. Annexin FITC-A, fluorescein-labeled annexin V; PI, propidium iodide. 2-A. Ionic Monomeric, 2-B. Ionic Dimeric, 2-C. Nonionic Monomeric, 2-D. Nonionic Dimeric, 2-E. Mannitol.

concentration increased, so did the number of necrotic cells compared with apoptotic cells for all experimental solutions. In addition, consistent with a lack of ionicity and monomer composition, the number of scattered dots decreased in Quadrant 1, 2, or 4 and increased in Quadrant 3 (Fig. 2 A–D).

Confocal images revealed that the untreated control contained predominantly live cells (green), whereas the number of dead cells (red) increased as the CM concentration increased (Fig. 3). In addition, there was a greater proportion of dead cells in the ionic monomeric CM preparation than in the non-ionic dimeric CM preparation (Fig. 3).

Discussion

Numerous studies have investigated the toxicity of CM in cell culture models, and various cell types including renal epithelial cells, endothelial cells, mesangial cells, pulmonary mast cells, smooth muscle cells, hepatic cells,

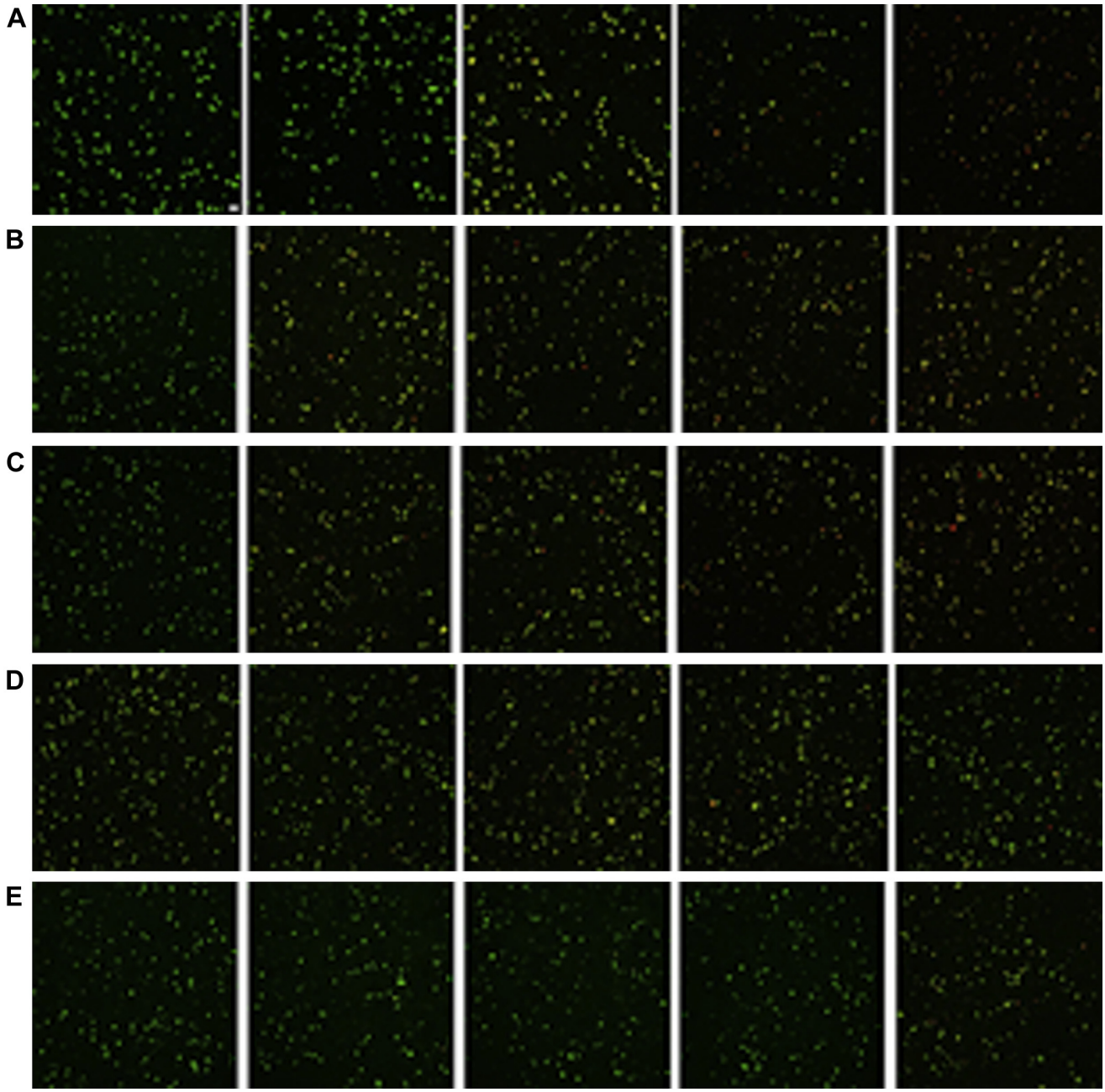

Fig. 3. Effects of different concentrations of various contrast media on nucleus pulposus cell viability. Cell death was determined using 5-chloromethylfluorescein diacetate (live cells, green) and propidium iodide (dead cells, red) staining and imaging under confocal microscopy. 3-A. Ionic Monomeric, 3-B. Ionic Dimeric, 3-C. Nonionic Monomeric, 3-D. Nonionic Dimeric, 3-E. Mannitol.

human fibroblasts, human neutrophils, and human embryonic kidney cells have been studied [5,13]. However, few studies have investigated contrast-induced cytotoxicity in human disc cells.

This is the first study to examine the impact of different physiochemical CM on human disc cell viability in vitro. Our findings demonstrate that, similar to a previous study of contrast medium nephrotoxicity, ionic CM is more toxic to human disc cells than non-ionic CM, whereas monomeric CM is more toxic than dimeric CM.

Although the toxic effects caused by iodinated CM are considered multifactorial, the properties of CM such as osmolarity and ionic strength could be the main determinants of cellular toxicity. Our study included all four groups of CM (non-ionic monomeric and dimeric, as well as ionic monomeric and dimeric), which have different osmolarities, molecular structures, and numbers of carboxyl groups (Table).

In terms of the ionic strength of CM, some studies found that ionic CM are more toxic than non-ionic CM [18–21]. However, other studies have not found such differences [9,12,22,23]. This discrepancy could be due to methodologic differences such as equiosmolar conditions or exclusion of melamine toxicity. The ionic strength of CM might be more important in vivo as indicated by the higher clinical nephrotoxicity of ionic CM compared with non-ionic CM.

Another potential determinant for the pathogenesis of cellular cytotoxicity is differences in osmolarity. As shown in Table, our experimental solutions had different osmolarities. The ionic monomeric CM was the most hyperosmolar followed by the ionic dimeric, non-ionic monomeric, and non-ionic dimeric CM. Hizoh et al. reported that ionic monomeric CM (diatrizoate) induced DNA fragmentation in Madin-Darby canine kidney cells more frequently than did non-ionic monomeric CM because of its hypertonicity [18]. However, a previous comparison of CM containing similar concentrations of iodine, similar to our study design, showed that CM with increased osmolarity induced greater cytotoxicity [23]. Schick CS et al reported that the osmolarity of CM seems to be responsible for toxic effects on the tight junction-associated membrane protein zonula occludens (ZO)-1 in renal epithelial cells [9].

In our study, ionic monomeric CM was the most hyperosmolar and was ultimately the most detrimental to cellular viability. Therefore, we used mannitol as a non-ionic hyperosmolarity control and it had the least toxic influence on human disc cell viability, which indicates that hyperosmolarity alone cannot explain our results. Comparative analyses between non-ionic CM versus ionic CM in rat renal cortical slices concluded that even under equiosmolar conditions, non-ionic CM were less nephrotoxic than ionic CM, which could be explained by gluconeogenesis and p-aminohippuric acid accumulation [24].

The chemotoxic effect of carboxyl groups, which are found only in ionic CM, could be one of the factors influencing disc cell cytotoxicity. Non-ionic CM have no carboxyl groups and have a higher number of hydroxyl groups than ionic CM, leading to decreased protein binding [13]. The chemical structure of CM may influence extracellular or cell membrane proteins and the few molecules of CM that enter cells could affect cell organelles or enzymes. For example, the carboxyl ion in an ionic CM is highly neurotoxic in the subarachnoid space [25,26]. In addition, ionic CM might cause ionic imbalance, which can influence protein function [27,28]. This could also explain why non-ionic CM is less toxic to human disc cells than ionic CM.

The comparison of monomeric CM with dimeric CM revealed that non-ionic dimeric CM was significantly less nephrotoxic than the monomeric, non-ionic CM in terms of renal hypoxia and urine hyperviscosity [29,30]. The essential goal in the development of dimeric CM is to reduce the osmolarity without decreasing the radiocontrast. Although iodine itself has well known cytotoxicity, the iodine concentrations in our experimental solutions were similar, thus this was not an important consideration in analyzing our results.

From the viewpoint of clinical application, dimeric CM has superior properties. Compared with a molecule of monomeric CM, which contains three atoms of iodine, each molecule of dimeric media contains six atoms of iodine. Thus, at equal iodine concentrations, a formulation of iotrolan contains half the amount of CM molecules than a monomeric CM formulation contains [14]. Dimeric non-ionic CM are more viscous than monomeric CM. There is evidence that dimeric non-ionic CM, which are more viscous than monomeric non-ionic CM, may lead to severe increases in the viscosity of urine in rats and increased viscosity may lead to mechanical stress [31].

All experimental solutions were toxic to NP cells in a dose-dependent manner. The actual CM concentrations during discography or percutaneous endoscopic lumbar discectomy were 300–320 mg iodine/mL of CM. Although the actual doses depend on the clinician, 1 mL would yield approximately 40.54 mg/mL CM if it homogeneously diffused (assuming that the volume of the intradiscal space is about 20 mL, whereas NP occupies about 40% of that space and contains about 80% water [32]). We used 0, 0.1, 1, 10, and 100 mg/mL CM preparations. We selected this range to include clinically relevant CM concentrations. CM will diffuse over time, thus the concentration will decrease. Therefore, examining cytotoxicity at low concentrations is still relevant.

There are some limitations to the present study. First, in vitro cytotoxicity may not adequately reflect in vivo conditions. The cytotoxicity of CM may differ according to the degree of degeneration, which is related to the chemical composition and histologic changes of the IVD. Because of these factors, the state of health of the NP cells will affect the analysis and interpretation of the results, and we cannot conclusively state that CM are cytotoxic in vivo. Further studies will help evaluate the clinical significance of CM with regard to cytotoxicity. In addition, the

mechanism through which CM is cleared from NP is unknown. The degree of cytotoxicity may depend on the even distribution of CM. Furthermore, we examined in vitro cytotoxicity at stable concentrations over 2 days, although in vivo the CM concentration may gradually decrease over time. Although the three-dimensional alginate beads provided a microenvironment similar to the physiologic three-dimensional matrix, it is difficult to exactly reproduce the in vivo structure of the disc.

Conclusions

The current in vitro study suggests that CM have a direct, dose-dependent cytotoxic effect on human disc cells. Non-ionic dimeric CM showed the least cytotoxic effect among the four different media tested. On the contrary, ionic monomeric CM had the most detrimental effect on disc cell viability. The in vitro differences in cytotoxicity between CM are associated with ionic strength, osmolarity, and viscosity.

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References

[1] Stout A. Discography. Phys Med Rehabil Clin N Am 2010;21:859–67.
[2] Lee H, Sowa G, Vo N, Vadala G, ÒConnell S, Studer R, et al. Effect of bupivacaine on intervertebral disc cell viability. Spine J 2010;10:159–66.
[3] Moon JH, Kuh SU, Park HS, Kim KH, Park JY, Chin DK, et al. Triamcinolone decreases bupivacaine toxicity to intervertebral disc cell in vitro. Spine J 2012;12:665–73.
[4] Wang D, Vo NV, Sowa GA, Hartman RA, Ngo K, Choe SR, et al. Bupivacaine decreases cell viability and matrix protein synthesis in an intervertebral disc organ model system. Spine J 2011;11:139–46.
[5] ten Dam MA, Wetzels JF. Toxicity of contrast media: an update. Neth J Med 2008;66:416–22.
[6] Haller C, Hizoh I. The cytotoxicity of iodinated radiocontrast agents on renal cells in vitro. Invest Radiol 2004;39:149–54.
[7] Fauser C, Ullisch EV, Kubler W, Haller C. Differential effects of radiocontrast agents on human umbilical vein endothelial cells: cytotoxicity and modulators of thrombogenicity. Eur J Med Res 2001;6:465–72.
[8] Haller C, Schick CS, Zorn M, Kubler W. Cytotoxicity of radiocontrast agents on polarized renal epithelial cell monolayers. Cardiovasc Res 1997;33:655–65.
[9] Schick CS, Haller C. Comparative cytotoxicity of ionic and non-ionic radiocontrast agents on MDCK cell monolayers in vitro. Nephrol Dial Transplant 1999;14:342–7.
[10] Kern MJ. Selection of radiocontrast media in cardiac catheterization: comparative physiology and clinical effects of nonionic monomeric and ionic dimeric formulations. Am Heart J 1991;122:195–201.
[11] Barrett BJ, Parfrey PS, Vavasour HM, ÒDea F, Kent G, Stone E. A comparison of nonionic, low-osmolality radiocontrast agents with ionic, high-osmolality agents during cardiac catheterization. N Engl J Med 1992;326:431–6.
[12] Heinrich MC, Kuhlmann MK, Grgic A, Heckmann M, Kramann B, Uder M. Cytotoxic effects of ionic high-osmolar, nonionic monomeric, and nonionic iso-osmolar dimeric iodinated contrast media on renal tubular cells in vitro. Radiology 2005;235:843–9.
[13] Sendeski MM. Pathophysiology of renal tissue damage by iodinated contrast media. Clin Exp Pharmacol Physiol 2011;38:292–9.
[14] Heinrich M, Schneider G, Grgic A, Humke U, Kramann B, Uber M. Effects of dimeric radiographic contrast medium iotrolan on Swine renal arteries: comparison with monomeric contrast media iohexol and iomeprol. Invest Radiol 2004;39:406–12.
[15] Aspelin P, Aubry P, Fransson SG, Strasser R, Willenbrock R, Berg KJ. Nephrotoxic effects in high-risk patients undergoing angiography. N Engl J Med 2003;348:491–9.
[16] Masuda K, Sah RL, Hejna MJ, Thonar EJ. A novel two-step method for the formation of tissue-engineered cartilage by mature bovine chondrocytes: the alginate-recovered-chondrocyte (ARC) method. J Orthop Res 2003;21:139–48.
[17] Benya PD. Modulation and reexpression of the chondrocyte phenotype; mediation by cell shape and microfilament modification. Pathol Immunopathol Res 1988;7:51–4.
[18] Hizoh I, Strater J, Schick CS, Kubler W, Haller C. Radiocontrast-induced DNA fragmentation of renal tubular cells in vitro: role of hypertonicity. Nephrol Dial Transplant 1998;13:911–8.
[19] Itoh Y, Sendo T, Yano T, Saito M, Kubota T, Oishi R. Comparison of cellular mechanisms underlying histamine release from rat mast cells induced by ionic and nonionic radiographic contrast media. Invest Radiol 2004;39:455–61.
[20] Fanning NF, Manning BJ, Buckley J, Redmond HP. Iodinated contrast media induce neutrophil apoptosis through a mitochondrial and caspase mediated pathway. Br J Radiol 2002;75:861–73.
[21] Grabowski EF. Effects of contrast media on endothelial cell monolayers under controlled flow conditions. Am J Cardiol 1989;64:10E–5E.
[22] Sumimura T, Sendo T, Itoh Y, Oka M, Oike M, Ito Y, et al. Calcium-dependent injury of human microvascular endothelial cells induced by a variety of iodinated radiographic contrast media. Invest Radiol 2003;38:366–74.
[23] Potier M, Lagroye I, Lakhdar B, Cambar J, Idee JM. Comparative cytotoxicity of low- and high-osmolar contrast media to human fibroblasts and rat mesangial cells in culture. Invest Radiol 1997;32:621–6.
[24] Nakamura M, Uozumi J, Soejima K, Kanou T, Tokuda N, Naito S. Inhibition of gluconeogenesis and p-aminohipuric acid accumulation in rat renal cortical slices by ionic and nonionic contrast media. Clin Exp Nephrol 2003;7:98–103.
[25] Dawson P. Chemotoxicity of contrast media and clinical adverse effects: a review. Invest Radiol 1985;20:S84–91.
[26] Almen T. Contrast media: the relation of chemical structure, animal toxicity and adverse clinical effects. Am J Cardiol 1990;66:2F–8F.
[27] Higgins CB. Mechanism of cardiovascular effects of contrast media: evidence for transient myocardial calcium ion imbalance. J Am Coll Cardiol 1985;6:854–5.
[28] Levin AR, Grossman H, Schubert ET, Winchester P, Gilladoga A. Effect of angiocardiography on fluid and electrolyte balance. Am J Roentgenol Radium Ther Nucl Med 1969;105:777–83.
[29] Seeliger E, Flemming B, Wronski T, ladwig M, Arakelyan K, Godes M, et al. Viscosity of contrast media perturbs renal hemodynamics. J Am Soc Nephrol 2007;18:2912–20.
[30] Seeliger E, Becker K, Ladwig M, Wronski T, Persson PB, Flemming B. Up to 50-fold increase in urine viscosity with iso-osmolar contrast media in the rat. Radiology 2010;256:406–14.
[31] Garvin JL, Hong NJ. Cellular stretch increases superoxide production in the thick ascending limb. Hypertension 2008;51:488–93.
[32] Roberts N, Hogg D, Whitehouse GH, Dangerfield P. Quantitative analysis of diurnal variation in volume and water content of lumbar intervertebral discs. Clin Anat 1998;11:1–8.