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Cytotoxicity of radiocontrast dyes in human umbilical cord mesenchymal stem cells

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ABSTRACT

Radiocontrast dyes are used for a wide range of diagnostic procedures for enhancing the image of anatomical structures, pain targets, and vascular uptake. While some of these dyes show toxicity to primary cells, their effect on stem cells, particularly mesenchymal stem cells (MSCs), is unknown. This study investigates the cytotoxic effects of two clinically used radiocontrast dyes, iohexol and iopamidol, on bone marrow and human umbilical cord MSCs. Exposure to these dyes significantly affected morphology of MSCs from both sources, as treated cells appeared transparent and no longer fibroblastoid. Cell viability decreased as determined by trypan blue and Annexin-V/PI staining, in a dose dependent manner with simultaneous loss of CD90 and CD105 concurrent with spontaneous differentiation in MSCs treated with iohexol and iopamidol. In addition, significantly higher cell death was observed in MSCs exposed to iopamidol than iohexol. At a concentration of 1:1, iohexol and iopamidol induced apoptosis in 19% and 92% ($< .01$) of MSCs, respectively. Global transcriptome analysis of treated MSCs revealed 139 and 384 differentially expressed genes in iohexol vs control and iopamidol vs control at $p \leq .01$ and 1.5-fold, respectively. This suggested that iopamidol had more significant effect on the transcription of MSCs. Based on these results a molecular mechanism of radiocontast dye induced cell death via intrinsic apoptosis pathway mediated by p53 was proposed. Since iopamidol was significantly more toxic than iohexol in human MSCs, a more careful examination of safety of radiocontrast dyes for clinical use is warranted.

1. Introduction

Radiocontrast dyes are routinely used in diagnostic procedures, including angiography, discography, gastrointestinal tract radiography, myleography, urography, and venography, as well as X-ray, computed tomography, and fluoroscopy (Bickham and Golembiewski, 2010; Andreucci et al., 2014a). These dyes enhance the image by distinguishing different anatomical structures, visualizing pain targets, vascular uptake, and contrast pattern flow (De Andrés Ares et al., 2014). Iodinated contrast dyes are among the most widely used, with about 75 million procedures performed yearly worldwide (Christiansen, 2005). Several reports have described harmful side effects of iodinated radiocontrast agents such as nausea, vomiting, hives, and fever (Munechika et al., 1998; Webb et al., 2003; Loh et al., 2010). Other studies showed that these radiocontrast dyes induced more severe effects such as renal impairment (Andreucci et al., 2014a), and thyroid dysfunction (Lee et al., 2015), as well as neurologic and cardiac toxicity (De Andrés Ares et al., 2014).

Based on the properties such as ionization, osmolality, and viscosity, iodinated radiocontrast dyes are grouped into four categories: ionic monomers (i.e. diatrizoate and metrizoate), ionic dimers (i.e ioxaglate), non-ionic monomers (i.e. iopamidol, iohexol, and iopromide), and non-ionic dimers (i.e. iodixanol and iotrolan) (Andreucci et al., 2014a; Spampinato et al., 2017). Non-ionic radiocontrast dyes have lower cytotoxicity and fewer adverse drug reactions (between 0.2% and 2.7% of cases) as compared to ionic dyes (Nordby et al., 1987; De Andrés Ares et al., 2014; Spampinato et al., 2017). Several in vitro studies have shown toxic effects of non-ionic radiocontrast dyes on primary cells including endothelial cells (Laerum, 1983; Ren et al., 2017), neutrophils (Fanning et al., 2002), proximal tubular cells (Shen et al., 2016), human embryonic kidney (HEK) cells (Romano et al., 2008), human fibroblasts, renal epithelial cells, smooth muscle cells, and hepatic cells (Kim et al., 2015). Contrast-induced nephropathy also caused a decrease in the number of endothelial progenitors in patients (Chiang

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et al., 2014). However, the effect of these dyes on adult stem cells such as mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs) is unknown. MSCs reside in supportive niches throughout the body and are responsible for the repair and regeneration of aged, injured, and diseased tissues or organs (Fuchs et al., 2004). MSCs are also capable of homing to distant sites upon injury or damage (Rustad and Gurtner, 2012). HSCs and MSCs isolated from bone marrow (BM) display limited growth in vitro (Zhironkina et al., 2012; Kumar and Geiger, 2017). Therefore, highly proliferative MSCs, particularly those isolated from umbilical cord are a good candidate for investigating the cytotoxic effects of radiocontrast dyes on adult stem cells.

We hypothesize that radiocontrast dyes could be more harmful or have a different effect on MSCs than primary cells. Some previous reports have shown high toxicity of radiocontrast dyes in primary cells (Laerum, 1983; Hardiek et al., 2001; Romano et al., 2008; Michael et al., 2014; Ren et al., 2017). Preliminary results of this investigation indicated more detrimental effects of radiocontrast dyes on MSCs than on the well-studied primary cell line, human embryonic kidney cells (HEK). These results emphasize the need for a more careful assessment of the clinical applications of these dyes.

2. Materials and methods

2.1. Maintenance and culture of MSCs

MSCs were isolated from umbilical cord samples and maintained according to the previously published protocols (Beeravolu et al., 2016, 2017). BM MSC and HEK293 cell lines were purchased from ATCC (Manassas, VA). Cells were grown in high glucose DMEM containing 10% FBS, 2 mM L-glutamine, 0.1% gentamicin, 0.2% streptomycin, and 0.12% penicillin in a 5% CO_2 incubator.

2.2. Treatment of cells with radiocontrast dyes

BM and UC MSCs (5×10^4 cells) and HEK (10^6 cells) were grown overnight in 6-well plates and then exposed to various concentrations ranging from 1:10–1:1 (dye: medium) of iohexol (24–120 mg I/ml; omnipaque-240, GE Health Care, Princeton, NJ) and iopamidol (37–185 mg I/ml; isovue-370, Bracco Diagnostics, Monroe, NJ). The cells were then investigated for morphological and biochemical changes resulting from exposure to radiocontrast dyes.

2.3. Cell viability and proliferation assays

Cells exposed to radiocontrast dyes for 24 h were dissociated with TrypLE (Life Technologies, Carlsbad, CA), centrifuged, and re-suspended in trypan blue solution and counted by hemocytometer to determine cell viability and proliferation. Cells stained blue were considered non-viable.

2.4. Immunophenotyping

To assess MSC surface markers, cells exposed to radiocontrast dyes were stained with FITC and APC conjugated antibodies against CD90, CD73, CD44, CD105 and CD29 and subjected to flow cytometric analysis using a FACS Canto II and Diva Software (Becton Dickinson, Franklin Lakes, NJ).

2.5. Immunostaining

For immunocytochemistry analysis, cells were fixed with 4% paraformaldehyde for 10 min at room temperature, permeabilized with 0.5% triton X-100 (Sigma, St. Louis, MO), and blocked with 2% bovine serum albumin (Sigma) for 1 h. Samples were incubated overnight with caspase 9 (1:200, Santa Cruz, Dallas, TX), washed in PBS, treated with FITC conjugated secondary antibody (1:200, Life technologies) for 2 h at room temperature, and counterstained with DAPI. Samples were then visualized using confocal microscopy (NIKON Instruments Inc., Melville, NY).

2.6. Western blot assay

Cells were lysed in RIPA buffer and protein was quantified using the Pierce 660 nm protein assay (Fisher Scientific, Pittsburgh, PA) on the NanoDrop 1000 spectrophotometer (Fisher Scientific). Equal amounts of protein were resolved on 12% SDS polyacrylamide gels and transferred to nitrocellulose membranes (BioRad, Hercules, CA). Membranes were blocked in 5% non-fat milk for 30 min at room temperature, and probed with 1:500 diluted caspase 9 antibody overnight at 4 °C. After washing, membranes were incubated with 1:10,000 diluted horseradish peroxidase conjugated secondary antibody (Santa Cruz) for 2 h at room temperature. Proteins were detected by using an ECL chemiluminescence kit and images were acquired using a ChemiDoc Touch Imaging System (BioRad). Finally, protein bands were analyzed using ImageJ (NIH, Bethesda, MA), and normalized to GAPDH.

2.7. Apoptosis analysis

MSCs (10^6) exposed to various concentrations of iopamidol and iohexol were suspended in 100 µl of Annexin-V binding buffer combined with 5 µl of Annexin-V/FITC (Biolegend, San Diego, CA) and 10 µl of propidium iodide (PI, Sigma) and analyzed by FACS. Cells that were Annexin-V negative and PI negative were considered viable. Cells only positive for Annexin-V were considered apoptotic and PI positive only cells were considered necrotic. Annexin-V and PI positive cells were late apoptotic.

2.8. Microarray analysis

MSCs treated with 1:4 concentration of iohexol and iopamidol were harvested and RNA was isolated from frozen cell pellets using the E.Z.N.A. Total RNA Kit I (Omega, Norcross, GA). RNA was purified using spin cartridge technology, quantified (Nanodrop 8000, Fisher Scientific), and stored at −80 °C. RNA was then amplified and labeled using the TargetAmp-Nano Labeling Kit (Epicenter, Madison, WI), which enables amplification and target preparation compatible with the Direct Hybridization Assay (Illumina, San Diego, CA). Amplification was performed with 500 ng of total RNA input following procedures described in the Target Amp-Nano Labeling Kit user guide. Hybridization and staining to the HumanHT-12 v4 Expression Bead Chip (Illumina) was performed using 750 ng of biotin-antisense RNA product following protocols outlined in the Whole-Genome Gene Expression Direct Hybridization Assay Guide. Subsequent scanning of the BeadChip was performed using the iScan System (Illumina). Gene expression data were imported into Illumina Genome Studio (v2011.1) and subsequently analyzed in Partek Genomics Suite (6.6 version 6.15.1207) and Pathway Studio (desktop version 11.0). The accession number for the microarray data concerning iohexol (OP) and iopamidol (IS) reported in this paper is NCBI GEO: GSE96043.

2.9. Quantitative real time polymerase chain reaction (qRT-PCR) analysis

Total cellular mRNA was isolated from cells using the GeneJET RNA purification Kit (Fisher Scientific) and purified by DNase treatment. cDNA was synthesized by using the iScript kit (BioRad) and qRT-PCR was performed in triplicate reactions using Sso-Advanced Universal SYBR Green Supermix Kit (BioRad) on the CFX96 Real-Time System (BioRad). Reference genes, *GAPDH* and *ACTIN*, were used to normalize the amplification of the target genes. Primer sequences are listed in Table 1.

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