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Influence of short-term iodinated radiographic contrast media exposure on reactive oxygen species levels in K562 cancer cells

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ABSTRACT

Background: Iodinated radiographic contrast media (IRCM) are commonly used for evaluating cancer diseases in diagnostic radiology. There are several studies that have showed the effects of IRCMs on various biological endpoints in normal cells. However, the effects of IRCMs on cancer cells is still a bit of a mystery.

Objectives: To investigate the effects of short-term iodinated radiographic contrast media exposure on reactive oxygen species levels in K562 cancer cells.

Materials and methods: Five commercially available IRCMs used were iohexol, iopamidol, iobitridol, ioxaglate, and iodixanol. A trypan blue exclusion assay was performed to evaluate the cytotoxicity of each IRCMs on K562 cancer cells. The effect of IRCMs on cell proliferation was further determined by counting the number of cells in metaphase. The reactive oxygen species (ROS) levels was determined at short-term by the use of a spectrofluorometric method.

Results: All IRCMs decreased in percentage of cell viability, number of metaphase cells, and levels of ROS in a concentration-dependent manner.

Conclusion: This study suggested that all IRCMs showed a short-term effect on K562 cancer cells by decreasing ROS levels in a concentration-dependent manner. In addition, IRCMs exhibited effect on cell viability and cell proliferation as well.

Introduction

Iodinated radiographic contrast media (IRCM) is a tri-iodinated derivative of benzoic acid.[1] IRCMs are the most commonly used methods in clinical practice for both diagnostic and therapeutic examinations. It can be involved in plain radiography, fluoroscopy, angiography, percutaneous cardiac and arterial interventions, and computed tomography (CT).[1, 2] The most common justifications for using contrast media is for evaluating cancer diseases. Injection of IRCMs are generally safe, however, there are notable adverse effects that are more likely to occur such as hypersensitivity reactions, contrast-induced nephropathy, and thyrotoxicosis.[3] In addition, results from many studies using a variety of biological endpoints have shown the effects due to exposure to IRCMs in cells and animal models.[4-8] However, information on such effects is mainly limited to only normal cells or animal models. Therefore, information on the potential risks from exposure to IRCM for cancer cells is lacking. As an initial step to fill this knowledge gap, we focused on erythromyelogenous leukemia cells line (K562) following short-term exposure to the IRCMs. Three biological endpoints (i.e.; cytotoxicity, a cell in metaphase, and reactive oxygen species)were determined in these studies. We used these biological endpoints due to the cytotoxicity and number of cells in metaphase which was referred to as toxicity and cell proliferation, respectively.Reactive oxygen species (ROS)are known to cause oxidative stress in several cellular molecules (i.e.; DNA, lipids, and proteins) and subcellular

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organelles (i.e.; mitochondria and plasma membranes).[9-12] Oxidative stress is one of the risk factors that play an important role in contrast-induced renal diseases.[13, 14]

Materials and methods

Chemicals

Five commercially available iodinated radiographic contrast media (IRCM) used were iohexol (omnipaque; GE Healthcare, China), iopamidol (iopamiro; Bracco, Italy), iobitridol (xenetix; Guerbet, France), ioxaglate (hexabrix; Guerbet, France), and iodixanol (visipaque; GE Healthcare, Ireland). These IRCMs are commonly used in diagnostic radiology.

Cancer cell and culture

Cancerous cell lines were erythromyelogenous leukemia cells line (K562). Cells were cultured in a tissue culture flask containing RPMI 1640 medium, supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin at 37 °C in a humidified 5% CO_2 atmosphere. The cell line cultures initiated a total of $1x10^5$ cells/mL before exponentially proliferating to a total of 8-10 x 10^5 cells/mL over 3 days. For the experiment, cultures were initiated at 5 x 10^5 cells/mL to obtain cells in the exponential growth phase to reach a total of about 8-10 x 10^5 cells/mL over 24 hours. Total number of viable cells was determined by a trypan blue exclusion assay. Total number of cells was determined by haemocytometer.

Cell viability

K562 cancer cells ($3x10^5$ cells/mL) were treated with IRCMs (10, 50, 100 mgI/mL) in 24-well plates at 37 °C for 72 hours. Total number of viable cells was determined by a trypan blue exclusion assay. Total number of cells was determined by haemocytometer. The percentage (%) of cell viability was calculated as followed;

% Cell viability = (Number of cells treated with IRCM/ Number of cells un-treated with IRCM)x100

Number of cells in metaphase

Number of cells in metaphase can be referred to as cell proliferation. K562 cancer cells ($3x10^5$ cells/mL) were treated with IRCMs (10, 50, 100 mgI/mL) in 24-well plates at 37 °C, 5% CO_2 in a humidified incubator for 72 hours. Next, 30 µL of 10 µg/mL colcemid was added to each well of the 24-well plates. After a 45 minutes incubation with colcemid, cells were washed with a phosphate buffer saline (PBS) and a total of 500 µL of 0.075 M KCl was added, followed by 45 minutes of additional incubation at 37°C, 5% CO_2 in a humidified incubator. Next, cells were washed with PBS and 5 mL of fixatives (Carnoy's solution, 3:1 v/v methanol: acetic acid) were added. Microscope slides were routinely at 4 °C until used for cell fixing. Fixed cells were dropped gently on clean microscope slides, were air-dried, and stained with a Wright Giemsa solution for 3 minutes. The number of metaphase cells was scored and recorded (Figure 1). For consistency, the microscopic analysis was performed by a single individual. Slides were coded so that the analyst was not aware of the treatment until after the slides were scored and the code was broken.

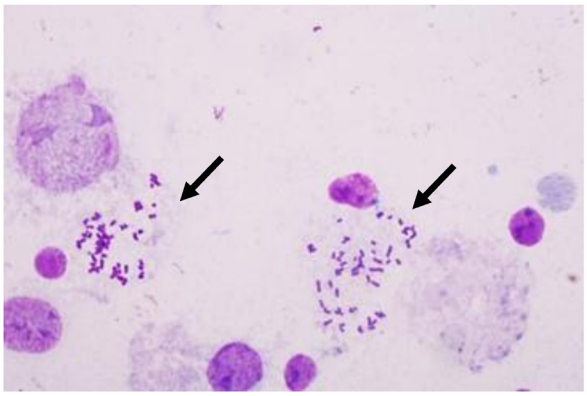

Figure 1. *Metaphase cells (Arrow). Magnification 100X*

Determination of reactive oxygen species (ROS) levels

Determination of reactive oxygen species levels was performed based on the work of Loetchutinat *et al*.[15] with some modifications. Briefly, a $1x10^5$ cells/mL suspended in HEPES-Na^+ buffer (pH 7.25) at 37 °C were treated with IRCMs (1, 10, 50 mgI/mL) for 5 minutes. That treated time is considered as short-term. After 100 seconds, 100 nM 2',7'-dichlorofluorescein diacetate (DCHF-DA) was then added into the system. Dichlorofluorescein (DCF) fluorescence intensity at 523 nm (excitation at 502 nm) was recorded as a function of time. Slope (dF/dt) of the tangent of the curve (experimental spectrofluorometric data) after time at the presence of DCHF-DA to 200 seconds was measured (Figure 2). The dF/dt was related to level of ROS. Thus, when dF/dt increases, it means that ROS levels are high. Conversely, when dF/dt has decreased, it means that ROS levels are low.

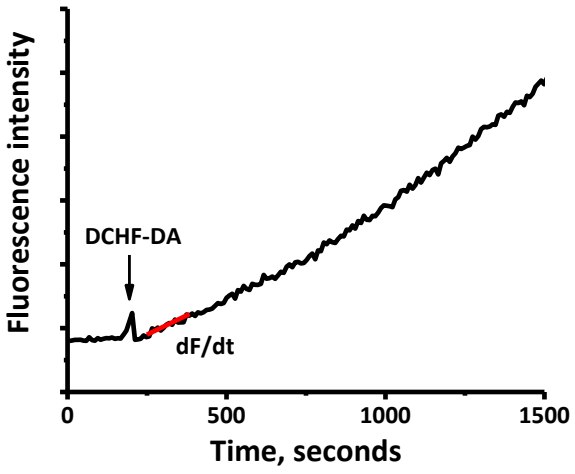

Figure 2. *Dichlorofluorescein (DCF) fluorescence intensity at 523 nm (excitation at 502 nm) as a function of time. Slope (dF/dt) of curve after time at the presence of DCHF-DA to 200 seconds.*

Statistical analysis

We presented the results as a mean±standard error of the mean (SE). Student's t-test was used independently to evaluate any statistical differences in the mean values between each test group and the corresponding control. A *p*-value of less than 0.05 was considered as statistically significant.

Results

Cell viability

Figure 3. shows the effects of IRCMs on K562 cancer cell viability. IRCMs decreased percentage of cell viability in a concentration-dependent manner. This result suggests that all IRCMs exhibited cytotoxicity on K562 cancer cells. However, four IRCMs (iodixanol, ioxaglate, iohexol, and iopamidol) significantly exhibited inhibition of cell viability at 50 and 100 mgI/mL when compared to a corresponding control. Iobitridol significantly exhibited inhibition of cell viability at 100 mgI/mL.

Metaphase cells

Figure 4. shows the number of cells in metaphase of K562 cancer cell after exposure to IRCMs. IRCMs reduced the number of metaphase cells in a concentration-dependent manner. However, all IRCMs except iopamidol significantly decreased the number of metaphase cells at 100 mgI/mL only when compared to a corresponding control. The result suggests that all IRCMs exhibited inhibition of K562 cancer cell proliferation.

Reactive oxygen species (ROS) levels

Figure 5. shows dF/dt of curve of K562 cancer cells after exposure to IRCMs. IRCMs reduced dF/dt of the curve in a concentration-dependent manner. However, all IRCMs except iodixanol significantly decreased dF/dt at 50 mgI/mL only when compared to a corresponding control. The result suggests that IRCMs could decrease ROS levels in K562 cancer cells.

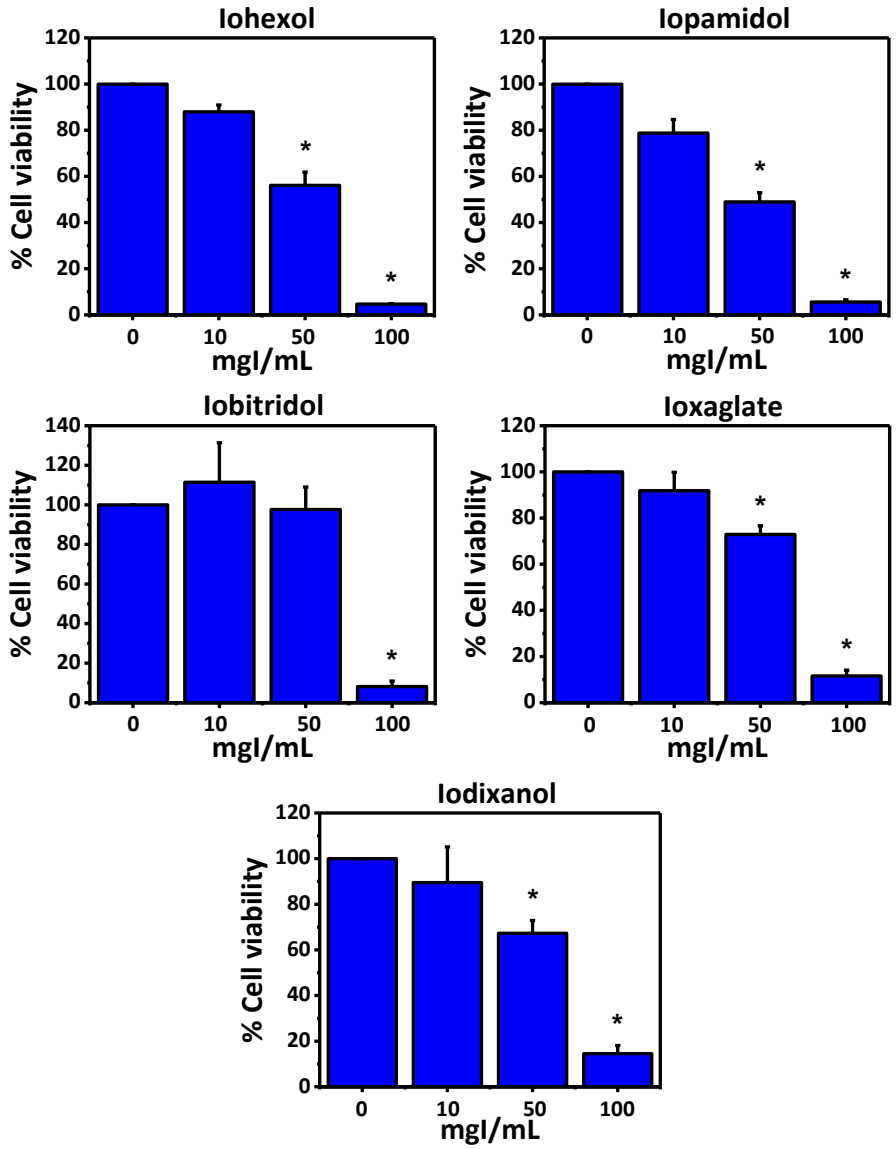

Figure 3. *Effects of IRCMs on K562 cancer cell viability. * p<0.05.*

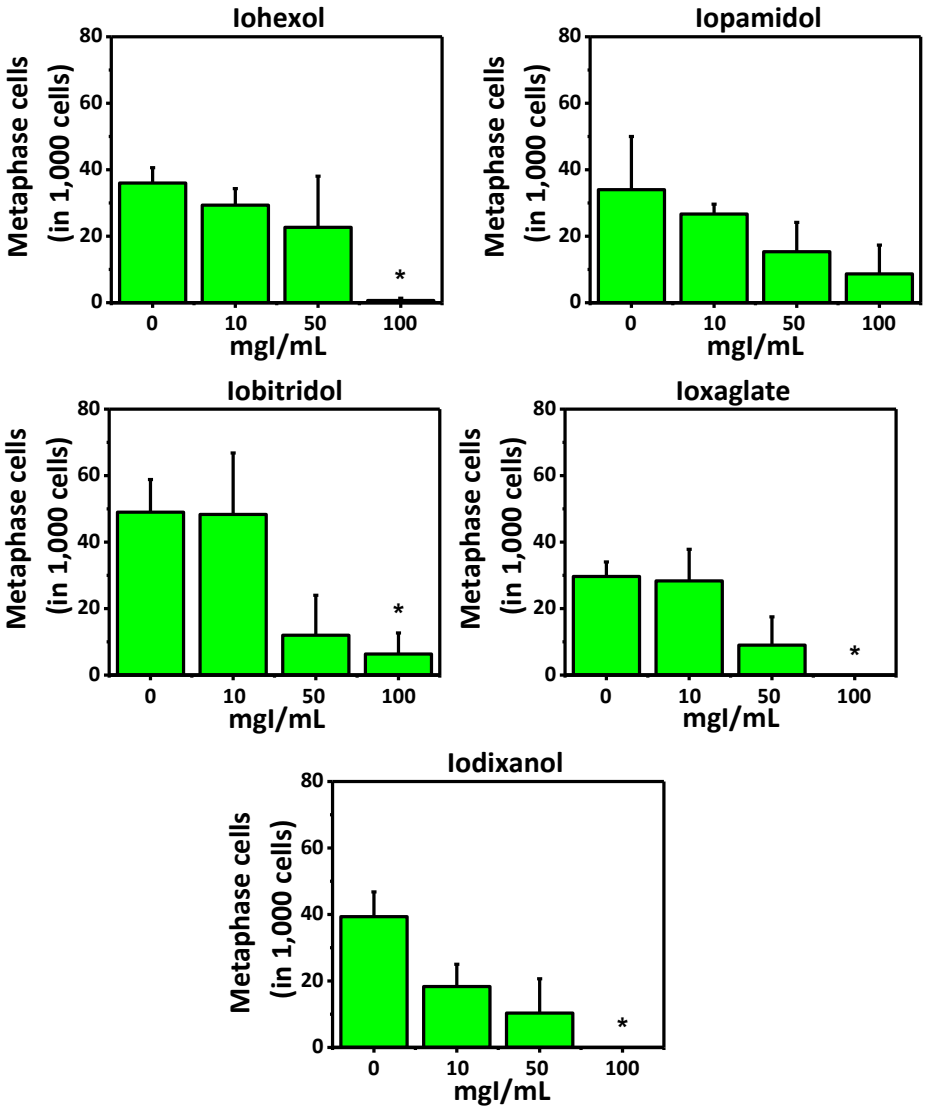

Figure 4. *Number of cells in metaphase in 1,000 cells of K562 cancer cells after exposure to IRCMs. * p<0.05.*

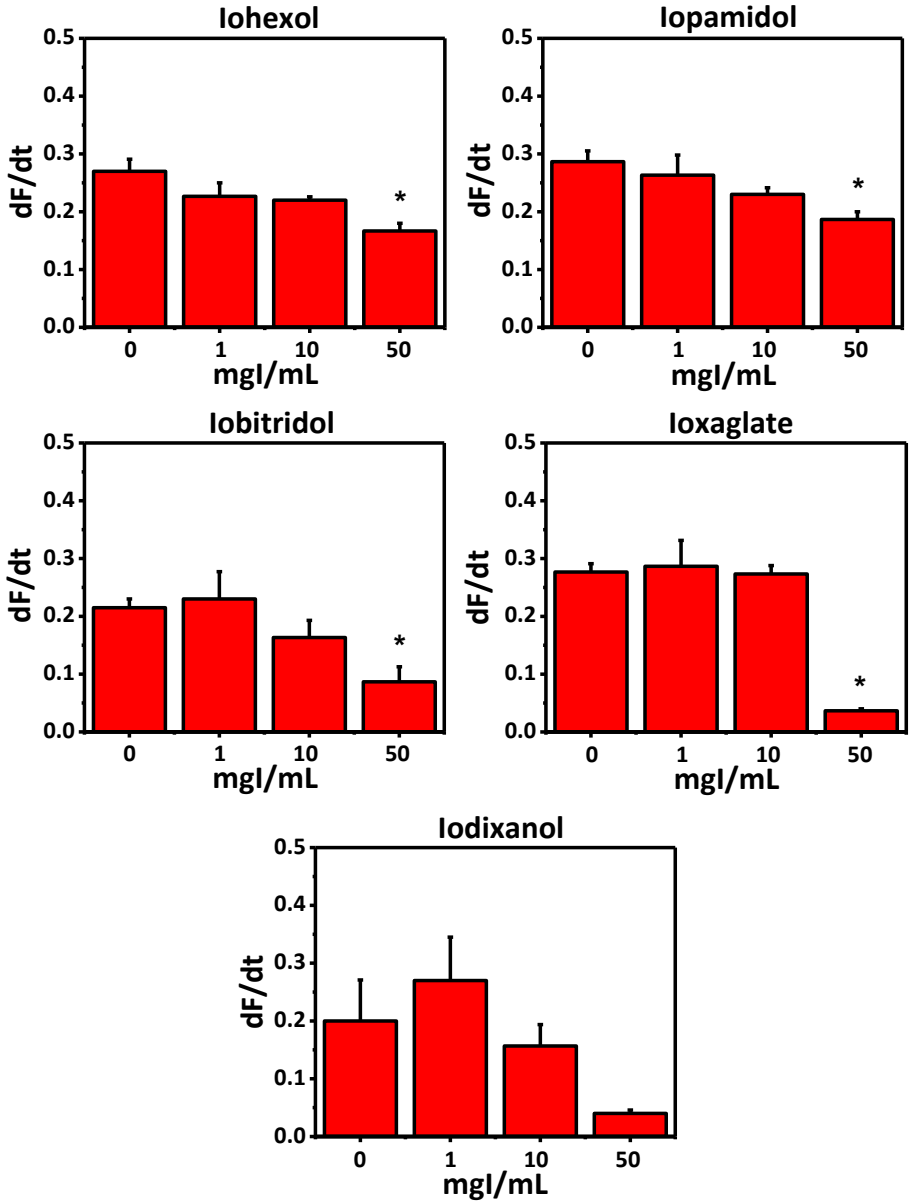

Figure 5. *The dF/dt of curve of K562 cancer cells after exposure to IRCMs. * p<0.05.*

Discussion

Oxidative stress resulting from an imbalance between free radicals and antioxidant agents, is one of the risk factors that plays an important role in IRCM-induced renal disease.[16] Our previously studies evaluated the potential properties of IRCMs (iohexol, iopamidol, iobitridol, ioxaglate, and iodixanol) *in vitro* free radical generating reactions. The results showed IRCMs exhibited weak *in vitro* antioxidant properties. This finding suggested that antioxidant ability depended on type of free radical production and concentration of IRCMs.[17] Our previous studies corresponded to the studies conducted by Berg *et al*.[18] These authors concluded that IRCMs (iodixanol, iohexol, ioxaglate, and diatrizoate) showed *in vitro* antioxidant properties in concentrations relevant for their clinical applications.[18] Furthermore, Xiong *et al*. observed increased intracellular ROS formation in renal tubular cells after exposure to IRCMs (ioversol). These studies suggested that ioversol induced renal tubular cell death in a concentration-dependent manner via an increase in oxidative stress.[19] In contrast, Zager *et al*. showed IRCM toxicity could be dissociated from tubular cell oxidant stress.[20] Current studies showed that IRCMs decreased ROS levels in K562 cancer cells in a concentration-dependent manner. Of note, Xiong *et al*. observed increased ROS levels in cells after exposure to IRCM for 1 hour[19] whereas the current studies observed decreased ROS levels occurring in cells after exposure to IRCMs for 5 minutes. It might be suggested that the effects of IRCMs was not only dependent on concentration but dependent on exposure time and cell type, as well.

Furthermore, our findings demonstrate that all IRCMs (iohexol, iopamidol, iobitridol, ioxaglate, and iodixanol) in present studies showed cytotoxicity and anti-proliferation effects on K562 cancer cells as being concentration-dependent

manner at a concentration of 10, 50, and 100 mgI/mL in ways similar to the studies conducted by Kim *et al*.[21] The authors investigated the effects of IRCMs (iodixanol, iopromide, ioxaglate, and ioxithalamate) on human disc cells. They showed human disc cells death had occurred in a concentration-dependent manner after exposure to IRCMs at a concentration of 0.1, 10, and 100 mg/mL.[21] It should be noted that there was a difference in the experimental design between the current studies and the studies conducted by Kim *et al*[21] in terms of the cell types used to investigate the effects of IRCMs. In the present studies, cancer cells were used instead of human disc cells in the studies conducted by Kim *et al*.[21] In addition, there are studies that have determined the effects of IRCMs on various cell types such as renal epithelial cells,[22, 23] endothelial cells,[23, 24] smooth muscle cells,[23, 25] human fibroblasts,[23, 26] and human neutrophils.[23, 27] Of note, toxic effects caused by IRCMs are considered multifactorial in that they can involve osmolarity and ionic strength.[23]

Conclusion

Taken together, we concluded that IRCMs such as iohexol, iopamidol, iobitridol, ioxaglate, and iodixanol showed short-term effects on K562 cancer cells by decreasing ROS levels in a concentration-dependent manner. In addition, IRCMs exhibited effect on cell viability and cell proliferation as well.

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Conflicts of Interest

The authors declare none of conflict of interest.

References

[1] Dickinson MC, Kam PC. Intravascular iodinated contrast media and the anaesthetist. Anaesthesia. 2008; 63(6): 626-34.

[2] Andreucci M, Solomon R, Tasanarong A. Side effects of radiographic contrast media: pathogenesis, risk factors, and prevention. Biomed Res Int. 2014; 2014: 741018.

[3] Thomson KR, Varma DK. Safe use of radiographic contrast media. Aust Prescr. 2010; 33: 4.

[4] Lee SY, Jang YH, Lee MY, Hwang J, Lee SH, Chon MK, et al. The effect of radiographic contrast media on reperfusion injury in the isolated rat heart. Korean Circ J. 2014; 44(6): 423-8.

[5] Kerl JM, Nguyen SA, Lazarchick J, Powell JW, Oswald MW, Alvi F, et al. Iodinated contrast media: effect of osmolarity and injection temperature on erythrocyte morphology in vitro. Acta Radiol. 2008; 49(3): 337-43.

[6] Cetin M, Devrim E, Serin Kilicoglu S, Erguder IB, Namuslu M, Cetin R, et al. Ionic high-osmolar contrast medium causes oxidant stress in kidney tissue: partial protective role of ascorbic acid. Ren Fail. 2008; 30(5): 567-72.

[7] Galtung HK, Loken M, Sakariassen KS. Effect of radiologic contrast media on cell volume regulation in rabbit proximal renal tubules. Acad Radiol. 2001; 8(5): 398-404.

[8] Galtung HK, Sorlundsengen V, Sakariassen KS, Benestad HB. Effect of radiologic contrast media on cell volume regulatory mechanisms in human red blood cells. Acad Radiol. 2002; 9(8): 878-85.

[9] Feinendegen LE, Pollycove M, Sondhaus CA. Responses to low doses of ionizing radiation in biological systems. Nonlinearity Biol Toxicol Med. 2004; 2(3): 143-71.

[10] Spitz DR, Azzam EI, Li JJ, Gius D. Metabolic oxidation/reduction reactions and cellular responses to ionizing radiation: a unifying concept in stress response biology. Cancer Metastasis Rev. 2004; 23(3-4): 311-22.

[11] Smith JT, Willey NJ, Hancock JT. Low dose ionizing radiation produces too few reactive oxygen species to directly affect antioxidant concentrations in cells. Biol Lett. 2012; 8(4): 594-7.

[12] Tungjai M, Phathakanon N, Rithidech KN. Effects of Medical Diagnostic Low-dose X Rays on Human Lymphocytes: Mitochondrial Membrane Potential, Apoptosis and Cell Cycle. Health Phys. 2017; 112(5): 458-64.

[13] Naziroglu M, Yoldas N, Uzgur EN, Kayan M. Role of contrast media on oxidative stress, Ca(2+) signaling and apoptosis in kidney. J Membr Biol. 2013; 246(2): 91-100.

[14] Persson PB, Tepel M. Contrast medium-induced nephropathy: the pathophysiology. Kidney Int Suppl. 2006(100): S8-10.

[15] Loetchutinat C, Kothan S, Dechsupa S, Meesungnoen J, Jay-Gerin J-P, Mankhetkorn S. Spectrofluorometric determination of intracellular levels of reactive oxygen species in drug-sensitive and drug-resistant cancer cells using the 2',7'-dichlorofluorescein diacetate assay. Radiation Physics and Chemistry. 2005; 72(2): 323-31.

[16] Bakris GL, Lass N, Gaber AO, Jones JD, Burnett JC, Jr. Radiocontrast medium-induced declines in renal function: a role for oxygen free radicals. Am J Physiol. 1990; 258(1 Pt 2): F115-20.

[17] Tungjai M, Sukantamala S, Malasaem P, Dechsupa N, Kothan S. An evaluation of the antioxidant properties of iodinated radiographic contrast media: An in vitro study. Toxicol Rep. 2018;5:840-5.

[18] Berg K, Skarra S, Bruvold M, Brurok H, Karlsson JO, Jynge P. Iodinated radiographic contrast media possess antioxidant properties in vitro. Acta Radiol. 2005; 46(8): 815-22.

[19] Xiong XL, Jia RH, Yang DP, Ding GH. Irbesartan attenuates contrast media-induced NRK-52E cells apoptosis. Pharmacol Res. 2006; 54(4): 253-60.

[20] Zager RA, Johnson AC, Hanson SY. Radiographic contrast media-induced tubular injury: evaluation of oxidant stress and plasma membrane integrity. Kidney Int. 2003; 64(1): 128-39.

[21] Kim KH, Park JY, Park HS, Kuh SU, Chin DK, Kim KS, et al. Which iodinated contrast media is the least cytotoxic to human disc cells? Spine J. 2015; 15(5): 1021-7.

[22] Andersen KJ, Christensen EI, Vik H. Effects of iodinated x-ray contrast media on renal epithelial cells in culture. Invest Radiol. 1994; 29(11): 955-62.

[23] Sendeski MM. Pathophysiology of renal tissue damage by iodinated contrast media. Clin Exp Pharmacol Physiol. 2011; 38(5): 292-9.

[24] Dascalu A, Peer A. Effects of radiologic contrast media on human endothelial and kidney cell lines: intracellular pH and cytotoxicity. Acad Radiol. 1994; 1(2): 145-50.

[25] Ribeiro L, de Assuncao e Silva F, Kurihara RS, Schor N, Mieko E, Higa S. Evaluation of the nitric oxide production in rat renal artery smooth muscle cells culture exposed to radiocontrast agents. Kidney Int. 2004; 65(2): 589-96.

[26] 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(10): 621-6.

[27] 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(899): 861-73.