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Cerium oxide nanoparticles sensitize non-small lung cancer cell to ionizing radiation

Shima Azizi [1], Arash Ghasemi [2], Hossein Asgarian-Omran [3,4], Zohreh Zal [1], Azadeh Montazeri [1], Hooman Yazdannejat [1], Seyed Jalal Hosseinimehr [1,*]

1 Department of Radiopharmacy, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

2 Department of Radiology and Radiation Oncology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

3 Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

4 Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

* Correspondence: sjhosseinim@yahoo.com, sjhosseinim@mazums.ac.ir (S.J.H); ORCID no: 0000-0001-8055-8036

Received: 22 October 2017; Revised: 5 November 2017; Accepted: 8 November 2017

ABSTRACT: Radiotherapy is an important strategy for cancer treatment, but resistance of tumor cells to ionizing radiation (IR) still remains a main challenging issue related to radiotherapy. The aim of this study was to evaluate the sensitizing effect of cerium oxide nanoparticles (CNPs) on non-small lung cancer (A-549) cells exposure to IR. A-549 cells were treated with CNPs and exposed to IR at dose 2 Gy. The radiosensitizing effect of CNPs was evaluated by clonogenic assay and flowcytometry. The findings of this study showed that CNPs reduced the frequencies of A-549 colony when these cells were exposed to IR. CNPs treatment prior exposure to IR significantly increased the IR-induced apoptotic incidences in A-549 cells. The present study demonstrates that CNPs to be an effective sensitizer on apoptosis and cell death induced by IR in A-549 cells.

KEYWORDS: Cerium oxide nanoparticle, nanoceria, radiosensitizing, apoptosis, ionizing radiation, radiosensitizer.

1. INTRODUCTION

Ionizing radiation (IR) is widely used for cancer treatment. In this strategy, IR is delivered to cancer cells and produces several toxic substances such as reactive oxygen species (ROS) and free radicals that have killing effects on cancer cells. Although IR induces apoptosis and death in cancerous cells, these cells may be resistant to IR. For beating on IR-resistance of cancerous cells, delivering of IR at high dose is recommended that results in side effects on normal tissue [1]. Since IR does not distinct between cancer and normal cells, IR induces side effects to normal tissues and it is usually a dose-limiting factor in radiation therapy [2]. The radiation-induced free radicals that attach to DNA sites resulted in chemically fixed damage in the presence of oxygen. This fixed DNA damage leading to an enhanced cell death. Then cells under hypoxia are more resistance to IR than normoxic cells. Tumor hypoxia is mainly caused by insufficient tumor angiogenesis and oxygen supply during tumor growth. Then oxygen content in cells is a crucial factor in radiosenstizing effect of IR on killing cells [3]. The biology of normal and tumor cells are different. ROS is mostly known to be involved in tumor induction and progression process but also enhance tumor cell radiosensitivity. The combined use of antioxidants and radiation enhance overproduction of ROS in tumor cells leading to enhanced radiosensitivity [4]. While the combined antioxidant with IR leading to protect normal cells against toxicity induced by IR [5, 6]. Radiosensitizers are used in radiation therapy for improvement of IR susceptibility of cancer cells. Radiotherapy can be applied at doses sufficiently low to minimize damage to neighbor normal tissues [7, 8]. Radiotherapy is an important treatment protocol for the

How to cite this article: Azizi S, Ghasemi A, Asgarian-Omran H, Zal Z, Montazeri A, Yazdannejat H, Hosseinimehr SJ. Cerium oxide nanoparticles sensitize non-small lung cancer cell to ionizing radiation. Marmara Pharm J. 2018; 22 (2): 307-313.

ISSN : 1309-0801

https://doi.org/10.12991/mpj.2018.68
Marmara Pharm J 2018; 22(2): 307-313

management of non-small cell lung cancer (NSCLC). However, radioresistance of cancer cells markedly impairs the effects of radiotherapy in patients. There is a great interest in the use of radiosensitizers that improve the cancer treatment with radiotherapy [9, 10]. Radiosensitzers are enabling to imbalance cellular oxygen and sensitize tumor cells to IR [11, 12]. CeO_2 cerium oxide nanoparticle (nanoceria, CNP) is developed as a therapeutic agent for oxidative stress associated diseases due to its antioxidant properties. Cerium is rare metal, when combined with oxygen, can form a particle that has highly contribution in oxidative stress process in cells. Some studies have shown CNP to possess anti-cancer effects [13-16]. CNP can change intracellular oxidative stress status that is partially attributed to the prooxidant activity of the CNP. Elevated ROS induces cell damages might eventually lead to the cancerous cell death [17]. CNPs inhibited the migration and proliferation of gastric cancer cells [14].

The ability of CNPs to modulation ROS could participate to their biological properties for improvement of radiotherapy in cancer treatment. Several studies exhibited that CNPs protected human normal cells against oxidative stress through antioxidant and anti-inflammatory process [18-21]. In radiotherapy regimen in patients with NSCLC, lung of patients is exposed to IR then normal and cancerous lung cells are encountered to free radicals and toxic substances results in cellular damages. It is clear that CNPs protect normal lung cells against oxidative stress; it is interesting to evaluate the effect of CNPs on radiation-induced toxicity in lung cancer cells [18].

Thus, the present study attempts to investigate the sensitizing effect of CNPs on IR-induced apoptosis and cell death in non-small cell lung cancer cells (A-549). These results demonstrate CNPs are potentially radiation sensitizer for the treatment of human lung cancer.

2.RESULTS

2.1.Effect of CNPs on the clonogenic survival of irradiated A-549 cells

The numbers of A-549 colonies in control and treated groups are shown in figure 1. Figure 2 shows a significant ($p < 0.001$) decrease in survival colony of irradiated A-549 cells (36% ± 6) as compared to the untreated control group (68% ± 5). On the other hand, when cells were treated to CNPs at concentration 50 µg/ml for three hours it showed a significantly reduction on the number of colonies (31% ± 6) as compared to control cells. CNPs treatment resulted insignificantly reduction on the survival clones in A-549 cells as compared to irradiated cells alone. Addition of CNPs to A-549 cells before exposure to IR resulted in a significantly reduction in survival clones (7% ± 1) as compared to irradiated cells and CNPs treated cells alones ($p < 0.001$) (Figure 2).

2.2. Apoptosis in A-549 cells treated with CNPs and radiation

To investigate the apoptotic effect of CNPs-induced radiosensitization, flow cytometry was used for measurement of apoptosis in A-549 (Figure 3). As shown in figure 4, compared to the control group (1.7% ± 0.4), the apoptosis rate of A-549 cells was increased in CPNs-alone group (4.1% ± 0.4) and IR-alone group (3.4% ± 0.2) ($P < 0.01$). It was observed an insignificantly difference between CPNs and IR groups in induction of apoptosis in A-549 cells. Furthermore, the apoptosis rate was significantly increased in CPNs + IR combination group (6.5% ± 0.5) compared to the IR alone and CPNs alone groups ($P < 0.05$).

3. DISCUSSION

This study demonstrated that CNPs have radiosensitizing effects on tumor cells by cell deaths and apoptosis induced by X-ray in A-549 cells. In this study, CNPs significantly reduced the number of colonies in irradiated A-549 cells. The number of colony was reduced to 7% in CNPs + IR group as compared to 31% and 36% with CNPs and IR alone. CNPs are known for their anticancer effects [22, 23]. CNPs change intracellular redox status which is partly attributed to the prooxidant activity of the CNPs in cancerous cells [24, 25]. Elevated ROS induces cell damages that might eventually lead to the cell death in cancerous cells treated with CNPs. CNPs induced the generation of ROS and malondialdehyde, and decreasing of superoxide dismutase and glutathione levels in human skin melanoma cells [25]. Mittal and Pandey reported that CNPs are enable to increase the intracellular ROS levels and induce DNA damage and cell deaths in A-549 cells [24]. Although, cancerous cell lines were sensitive to CNPs treatment, normal cell lines (keratinocytes and fibroblasts cells) were insensitive [17].

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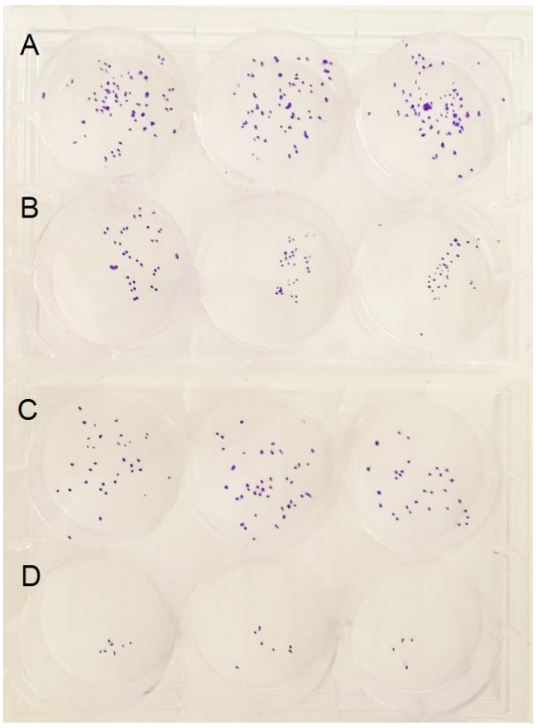

Figure 1. Effect of cerium oxide nanoparticles (CNPs) on the clonogenic survival of irradiated A-549 cells. Cells were treated with CNPs at concentration 50 µg/ml for three hours and irradiated with X-rays at dose 2 Gy. A; control, B: CNPs, C: ionizing radiation (IR), D: CNPs + IR.

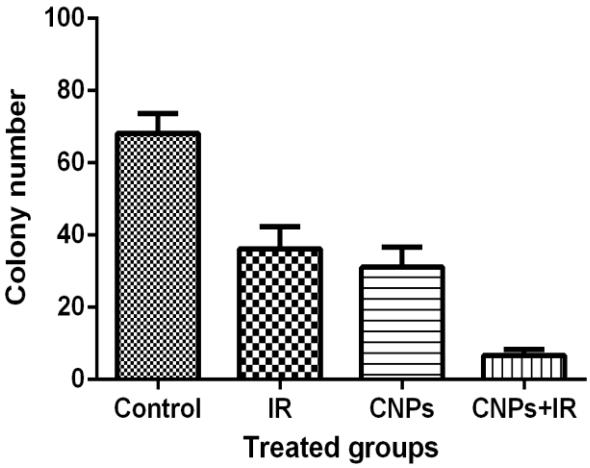

Figure 2. Effect of cerium oxide nanoparticles (CNPs) on the clonogenic survival of irradiated A-549 cells. Cells were treated with CNPs at concentration 50 µg/ml for three hours and irradiated with X-rays at dose 2 Gy. At the end of 15 day incubation, survival colonies from each group were scored. One hundred cells were seeded cells and then numbers of colonies were counted. Values were mean ± SD (N = 3).

Colon *et al*. reported that CNPs protected the gastrointestinal epithelium against radiation-induced damage through acting as free-radical scavengers and increasing the production of superoxide dismutase [26]. Other studies showed the radioprotective effects of CNPs in human normal cells and animal model [27, 28]. CNPs exhibited antioxidant properties through different mechanisms such as free radical scavenging, reducing ROS level and increase endogenous antioxidants [19, 26, 29]. These antioxidant mechanisms are associated to protection cells against DNA damage and deaths induced by IR. The antioxidant activities of CNPs are responsible for its radioprotection. CNPs display minimal toxicity to normal tissues and provide protection from various forms of oxidative stress disorders [23]. In normal lung cells, CNPs have cytoprotective and antioxidative effects [18].

These nano particles prevented normal epithelium oxidation and inhibit inflammatory process [18, 20, 21]. However CNPs have antioxidant activity and anti-inflammatory activities, these effects are leading different results in normal and cancerous cells. Several studies demonstrated that antioxidants have radioprotective effects on normal cells by mainly decreases ROS levels while these compounds have radiosensitive effects in tumor cells by increasing ROS levels. Curcumin as a powerful antioxidant has radioprotective effect on normal cells [30], while it has radiosensitizing effect in tumor cells by increasing ROS level and suppressing inflammatory process such as NF-κB activation pathway induced by ionizing radiation [31-33]. Genestein has antioxidant activity and protect healthy cell against toxicity induced by IR [34, 35]. Genistein stimulated irradiation-induced intracellular reactive oxygene and suppressed irradiation-induced COX-2 expression, as an inflammation marker, resulted in radiosensitizing effect in cancerous cells [36]. The combined with radiation, genistein increased the ROS levels in A-549 cells and significantly increased cell apoptosis in A-549 [37]. The dual capabilities of CNPs to act as an oxidant in cancer cells, yet antioxidant in normal cells, supports the role of CNPs as an promising agent for radiotherapy that could significantly improve cancer treatment.

https://doi.org/10.12991/mpj.2018.68
Marmara Pharm J 2018; 22(2): 307-313

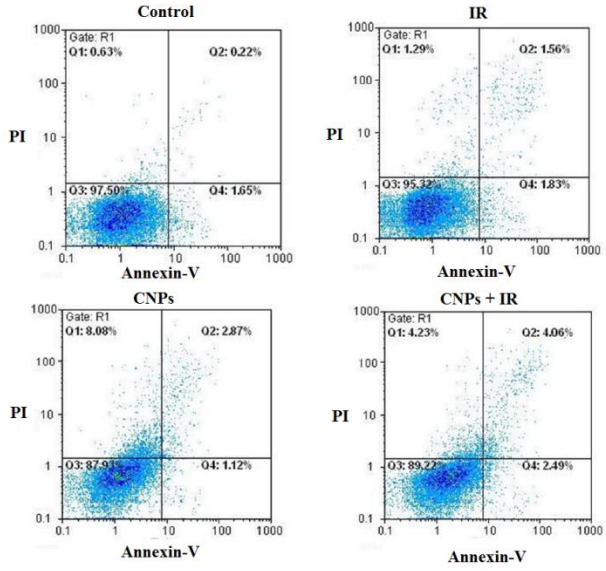

Figure 3. Effect of cerium oxide nanoparticles (CNPs; 50µg/ml) on radiation-induced apoptosis in A-549 cells. Cells were analyzed for Annexin V binding and for PI uptake using flow cytometry. Representative dot plots of one set of five independent experiments of Annexin V and PI staining is shown. In each figure, the lower left quadrant (Annexin V^- and PI^-) was considered as live cells,the lowerright quadrant (Annexin V^+ and PI^-) was considered as apoptotic cells, the upper right quadrant (Annexin V^+ and PI^+) was considered as late apoprotic and necrotic cells (N =3).

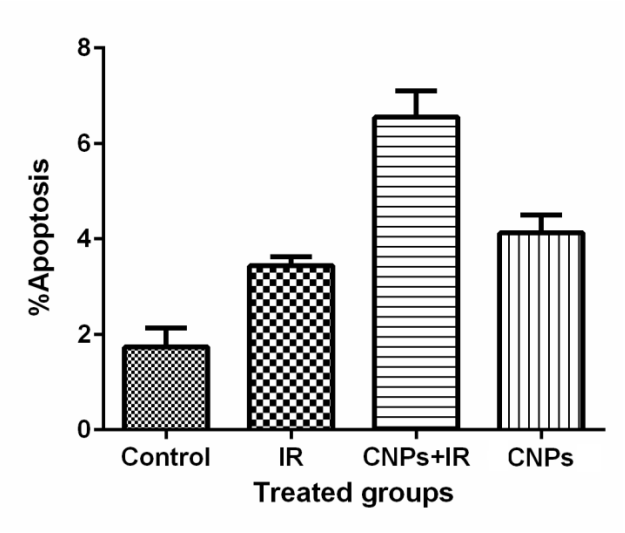

Figure 4. Effect of cerium oxide nanoparticles (CNPs; 50 µg/ml) on ionizing radiation (IR)-induced apoptosis in A-549 cells. The percentages of apoptotic cells were shown in experimental groups. Values are expressed as mean ± SD of three experiments. $P< 0.05$: 2 Gy group alone compared to sham control, CNPs+ 2 Gy and CNPs groups.

4. CONCLUSION

We demonstrated that the cerium oxide nanoparticles sensitized human lung cancer A-549 cells to radiation-induced apoptosis and death. This result provides a new indication of CNPs for sensitizing of A-549 cancer cells to radiation therapy in patients. Since the protection of normal cell against toxicity induced by IR, future study is necessary to perform for possible radioprotection of CNPs on healthy cell.

5. MATERIAL AND METHODS

5.1. Materials

A-549 cell line was obtained from the National Center for Genetic and biological reserves of Iran and cultured at 37 °C and 5% CO_2 in. Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Paisley, UK) supplemented with 10% fetal bovine serum (FBS) (Gibco, UK) and 100 µg/mL penicillin–streptomycin (Gibco, UK). Annexin-V-FLUOS Staining Kit was obtained from eBioscienc (USA). Cerium oxide nanoparticle was obtained from Neutrino Co., (Iran). Particle size was 378 nm, with polydispersity index (PDI) 0.372 and Zeta -7.11 that were determined with Zetasizer 3600 Nano ZS (Malvern Instrument Ltd, Malvern, UK).

5.2. Clonogenic assay

A-549 cells were plated in 6-well dishes (100 cells in dish), grown for 24 hours and then treated with CNPs (50 µg/ml) for three hours. This concentration of CNPs was selected from other study that CNPs were not

exhibited any toxicity on epithelial normal lung cell line (BEAS-2B) during 24 and 48 h exposure to these particles at concentration 50 µg/ml [18]. The CNPs-treated cells were exposed to IR at dose of 2 Gy with 6 MV X-ray beam produced by a Linear accelerator (Siemens, Primus, Germany) at a dose rate of 1.9 Gy/min. After exposure to IR, cells were incubated in complete culture medium for up to 15 days, colonies were washed with 1× PBS and then were fixed with 1% glutaraldehyde for 30 min and were stained with 0.5% crystal violet (wt/vol) in water. The colony containing at least 50 cells was scored and considered to cell alive. The other groups as control (without CNPs treatment and exposure to IR), CNPs (treatment with CNPs, without exposure to IR) and IR (only exposure to IR) were treated at same manner to above protocol.

5.3. Apoptosis determination by flow cytometry

Apoptotic cells were analyzed 24 h after exposure of cells to IR. A-549 cells (2×10^5) were treated with CPNs at concentration 50 µg/ml for three hours in 12-well plates and then were exposed to IR at dose 2 Gy. Cells were cultured in DMEM medium for 24 h. Apoptosis were determined using an 'Annexin-V-FLUOS Staining Kit' according to the manufacturer's instruction (eBioscience, USA). Briefly, cells were washed with PBS and incubated with Annexin-V FLUOS labeling solution (containing 2 µL Annexin-V-FLUOS labeling reagent and 2 µL propidium iodide (PI) solution in 100 µL incubation buffer for each sample) at room temperature in the dark for 15 min. Following incubation, analysis was performed with a Partec flow cytometer system and Flomax software (Partec, Germany). Non-stained cells were used as negative control for background determination. For each sample, a minimum of 10000 events were counted and analyzed.

5.4. Statistical analysis

The data values are presented as means ± standard division (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), as well as Tukey's multiple comparison and Dunnett multiple comparison tests. *P* value <0.05 was considered as significant and highly significant (Prism 7 Software, 2016, USA).

Acknowledgments: This study was supported by a grant from Mazandaran University of Medical Sciences, Sari, Iran. Seyed Jalal Hosseinimehr was received this grant.

Author contributions: Concept - S.J.H.; Design - S.J.H., S.A., H.S.O.; Supervision – S.J.H.; Resource – S.J.H.; Materials – S.A.; Data Collection &/or Processing – S.A., A.G., H.A.O., Z.Z., A. M., H.Y.; Analysis &/or Interpretation – S.A., H.A.O., S.J.H.; Literature Search – S.A., S.J.H.; Writing – S.J.H; Critical Reviews – S.A., A.G., S.J.H., H.A.O., Z.Z., H.Y., A.M.

Conflict of Interest: All Authors reported no potential conflict of interest relevant to this article.

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