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Mitigation of radiation-induced pneumonitis in mice using alpha-tocopherol and nano-micelle curcumin

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ABSTRACT

Pneumonitis is one of the most common consequences of exposure to high doses of ionizing radiation in lung tissue. It is associated with acute inflammation and infiltration of inflammatory cells, leading to massive release of several inflammatory mediators. Some studies have reported a risk for pneumonitis following exposure to a radiation disaster. In this experimental study, we aimed to evaluate possible mitigatory effect of alpha-tocopherol and nano-micelle curcumin on radiation pneumonitis in mice. 30 male mice were divided into 6 groups, including control, alpha-tocopherol or nano-micelle curcumin treatment, radiation, and radiation plus alpha-tocopherol or nano-micelle curcumin. Treatments were initially performed 1 day after irradiation and continued for 1 month. Irradiation was performed with 18 Gy using a cobalt-60 gamma rays source. After 8 weeks, mice were sacrificed and the lung tissues were removed for histopathological evaluation. Our study showed that pneumonitis was associated with was able to reduce inflammatory cells infiltration, edema, vascular and alveolar damage. Treatment with alpha-tocopherol could attenuated inflammation markers, while it could not mitigate vascular and alveolar damage. By contrast, nano-micelle curcumin was able to reduce inflammation, and vascular and alveolar damage. This study revealed that treatment with alpha-tocopherol or nano-micelle curcumin can mitigate radiation-induced pneumonitis in mice. These findings may pave the way to mitigation of radiation toxicity after a radiation disaster such as nuclear explosion or radiological events.

KEY WORDS: RADIATION; LUNG; PNEUMONITIS; ALPHA-TOCOPHEROL; NANO-MICELLES CURCUMIN

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INTRODUCTION

Pneumonitis is the most common frequent side effect of high dose ionizing radiation in lung tissue. It is characterized by acute inflammation in the arisen from inflammatory cells infiltration and thereby the release of several inflammatory mediators. A high dose of ionizing radiation has been shown to induce massive cell death followed by release of cytokines, inflammatory mediators, and reactive oxygen and nitrogen species (ROSs and RNSs, respectively). Chronic increased level of cytokines and production of free radicals lead to alveolar, vascular, bronchiolar damage, thereby interrupting normal function of lungs. This phenomenon may be observed in patients with various types of cancer in thoracic area such as non-small cell lung carcinoma (NSCLC), breast cancer, bronchial cancer, and etc. Moreover, mortality has been shown in association with lung injury among radioactive iodine- treated thyroid cancer patients with metastasis in the lungs, (Kwa et al. 1998, Rodrigues et al. 2004, Tsoutsou Koukourakis 2006, Hebestreit et al. 2011 and Najafı et al., 2018).

In addition to cancer patients, a body of evidence have suggested that pneumonitis may occur among people who were already exposed to acute ionizing radiation during nuclear or radiological disaster. Although bone marrow and gastrointestinal tract toxicity are the most common culprits for death following a radiation disaster, some studies have proposed that during whole body exposure to ionizing radiation, lung tissue may receive more than 7-8Gy; while lower parts of body may be affected by non-lethal doses, (Christofidou-Solomidou et al. 2011; Christofidou-Solomidou et al. 2017 and Yahyapour et al., 2018a).

Fortunately, stem cell therapy is able to prevent death caused by hematopoietic and gastrointestinal tract toxicity that occur following exposure to an acute radiation dose greater than 8Gy (Lataillade et al. 2007). Although this worthwhile therapeutic strategy can prevent early death, organ failure in lung following exposure to radiation is likely to occur. Additionally, there is piece of evidence indicating death caused by lung toxicity among people who were exposed to Chernobyl nuclear station explosion, (Deas et al. 2017; Yahyapour et al. 2018c).

Experimental studies have proposed that treatment with some antioxidants and immunomodulatory agents may mitigate radiation injury in radiosensitive organs. So, identification of mitigatory effect of low toxic antioxidants and flavonoids may be useful for to address the possible radiation disasters. Curcumin is a well-known herbal agent with potent anti-inflammatory and antioxidant effects. Previous studies have shown considerable protective effect of curcumin against toxicity of ionizing radiation, (Jurenka 2009, Cho et al. 2013; Patil et al. 2015 Yahyapour et al. 2018b Bagheri et al. 2018).

One of the most important concerns about the curcumin is its low absorption in gastric owing to high lipophilicity. This issue has been tackled in nano-micelle form of curcumin (Li et al. 2015). Consequently, more efficiency can be achieved with the same dose following treatment with nano-micelle form of curcumin. Alpha-tocopherol is another antioxidant that was used for mitigation of lung pneumonitis in the current study. It has shown a potent antioxidant effect which is able to protect and mitigate radiation toxicity (Ferreira et al. 2004; Singh et al. 2010). In this study, we aimed to illustrate possible mitigatory effect of nano-micelle form of curcumin and alpha-tocopherol on radiation-induced pneumonitis.

MATERIAL AND METHODS

Experimental design

All 30 male mice, purchased from Razi institute, Tehran, Iran; were kept under standard condition (temperature = 25C, humidity=55%, 12h light/12h dark). Then, they were divided into 6 groups (5mice in each). 1) G1 (control): mice who did not receive any drug or irradiation, and were taken only anesthesia drugs in a similar dose as other groups; 2) G2 (irradiation): mice who were irradiated locally in chest area, 3) G3 (alpha-tocopherol treatment): those received 200mg/kg/day alpha-tocopherol, 4) G4 (curcumin treatment): mice in this group were received 100mg/kg/day nano-micelle form of curcumin, 5) G5 (radiation plus alpha-tocopherol): mice were exposed to gamma rays, and after 24h, treatment with alpha-tocopherol was started, and 6) G6 (radiation plus curcumin): mice were exposed to gamma rays, and after 24h, treatment with nano-micelle form of curcumin was started. Finally, all mice were sacrificed 8weeks after irradiation and then the lung tissues were removed for histological evaluation.

Irradiation and drug administration

Alpha-tocopherol soft gel capsule was obtained from Nature Made and dissolved in olive oil. Each mouse orally received 1ml containing 6mg alpha-tocopherol (equal 200mg/kg). Then, treatment with alpha-tocopherol was started 1day after irradiation with the procedure of 5-time per week for 4 weeks. Nano-micelle form of curcumin was purchased from Exir Nano Sina, Mashahd, Iran, and was dissolved in water at a concentration of 1mg/ml. As mice drink 3 milliliter water daily which was equal to 100mg per kg per day. Treatment was started 24h after irradiation and continued for 1 month. Then, Irradiation was performed using a cobalt-60 source. Before this, mice were anesthetized using a combination of ketamine and xylazine (). After that, they were exposed to gamma rays source at supine position with

Table 1. Histopathological results of pneumonitis incidence following mice lung exposure to ionizing radiation and mitigation by alpha-tocopherol and nano-micelle curcumin.

	Macrophage infiltration	Lymphocyte infiltration	Neutrophil infiltration	Vascular thickening	Alveolar thickening	Edema and thrombosis
Control	0±00	0.50±0.50	0±00	0±00	0±00	0±00
Alpha-tocopherol	0±00	0.66±0.47	0±00	0±00	0±00	0±00
Curcumin	0±00	0.33±0.47	0±00	0.66±47	0±00	0±00
Radiation	3.0±00	3.0±00	3.0±00	2.0±00	1.66±47	1±00
Radiation plus Alpha-tocopherol	0.6±0.49	1.2±0.40	0.40±0.48	1.4±0.80	1.4±0.80	0.6±0.49
Radiation plus Curcumin	1.0±1.41	1.0±0.81	0.33±0.47	0.33±0.47	0.33±0.47	0±00

a distance of 80cm. subsequently, irradiation of chest was done at a dose of 60cGy/min and other parts of the mice's body were shielded using lead block.

Histopathological evaluation

Following fixation of lung tissues, all samples were embedded into a block of paraffin. Then, they were cut by microtome to pieces with 4-micron thickness. Resultant lung pieces were located on the slides to be stained. Each slide was stained using hematoxylin and eosin (H&E). The stained slides were evaluated for morphological changes by a histopathologist. Pneumonitis markers including infiltration of inflammatory cells and alveolar and vascular damages were detected. All these procedures were performed in the pathology unit of Imam Khomeini hospital, Tehran University of Medical Sciences, Tehran, Iran.

Statically analyses

Results of histopathological evaluation were reported as grade 0 to3. Changes in each groups were calculated as mean ± standard deviation. Mann-Whitney test was used to evaluate the significance in the groups (SPSS V16).

RESULTS

Histopathological evaluations showed a significant increase in pneumonitis markers following exposure to radiation. a drastic increase was also shown in the infiltration of inflammatory cells including macrophages,

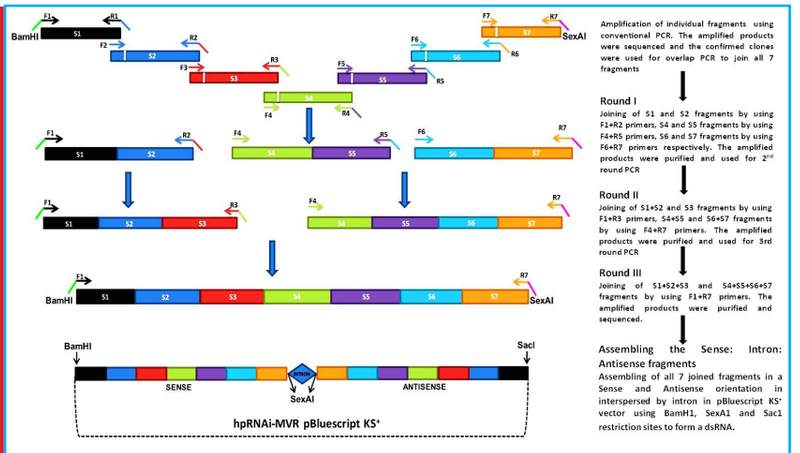

FIGURE 1. Histopathological evaluation of radiomitigatory effects of alpha-tocopherol and nano-micelle curcumin on radiation-induced pneumonitis. Irradiation cause severe infiltration of macrophages, neutrophils and lymphocytes; as well as vascular and alveolar thickening, and edema. However, treatment with nano-micelle curcumin or alpha-tocopherol attenuated these markers significantly. A: Control; B: Nano-micelle curcumin; C: alpha-tocopherol; D: Radiation; E: Radiation + Nano-micelle curcumin; F: Radiation + alpha-tocopherol (H&E staining, ×100).

lymphocytes and neutrophils (p=0.008). Treatment with alpha-tocopherol caused significant amelioration of these inflammatory cells (p value of 0.018, 0.014, and 0.018 for mitigation of macrophages, lymphocytes, and neutrophils, respectively). When mice were treated with alpha-tocopherol after irradiation, only a low infiltration of lymphocytes observed, with infiltration of macrophages and neutrophils being similar to that in control group. Irradiation also lead to a moderate vascular thickening (p=0.008) and a mild alveolar thickening (p=0.01) and edema (p=0.008). However, treatment with alpha-tocopherol failed mitigate these parameters. Treatment with alpha-tocopherol alone did not cause any toxicity, and lung morphology in this group was similar to that in control group.

Treatment with curcumin nano-micelle has a potent mitigatory effect on radiation-induced pneumonitis. However, treatment with neither nano-micelle curcumin nor alpha-tocopherol led to significant change in morphological properties of mice lung tissue. On the other hand, treatment with nano-micelle curcumin after irradiation could significantly attenuate infiltration of lymphocytes (p=0.037) and neutrophils (p=0.037), however, the difference in infiltration of macrophages wasn't significant. administration of this drug also led to a remarkable attenuation of vascular thickening (p=0.037), edema, and thrombosis (p=0.025). However, difference in alveolar thickening between radiation group and radiation plus nano-micelle curcumin was not significant.

DISCUSSION

As previously mentioned, radiation-induced pneumonitis is the acute response of lung tissue which leads to respiratory impairments, and also may cause death in a few months. As results of this study showed, lung exposure to ionizing radiation caused severe infiltration of macrophages, lymphocytes and neutrophils, all of which resulting from massive DNA damage and cell death which in turn lead to the release of chemokines and recruitment of circulatory cells into the injured area (Mukaida et al. 1998). These cells are able to produce massive free radicals via a phenomenon named respiratory burst. Production of free radicals activates several signaling pathways, including redox reactions by pro-oxidant enzymes, that lead to chronic oxidative stress (Yahyapour et al. 2018d). Also, they are capable of releasing several pro-inflammatory and pro-fibrotic cytokines. Interactions between oxidative stress and inflammatory cytokines plays a key role in development of late effects of radiotherapy and radiation disaster. targeting these interactions has been proposed as a strategy for mitigation of radiation injury (Farhood et al. 2018). With respect to the results, post irradiation-chronic inflammation causes alveolar and vascular injury, edema, and thrombosis.

To date, some agents have been proposed for mitigation of radiation-induced lung injury in animal models. Targeting COX-2 by celecoxib has been shown to alleviate pneumonitis and increase survival following local chest irradiation (Hunter et al. 2013). renin-angiotensin system also has been proposed as a target for mitigation of lung pneumonitis (Medhora et al. 2012). Ghosh et al. showed that administration of captopril or losartan (which are angiotensin inhibitor) mitigates radiation pneumonitis and increases survival through the improved breathing, amelioration of vascular injury, and reduction of infiltration of inflammatory cells (Ghosh et al. 2009; Molthen et al. 2012). Similar results have been obtained with antioxidants. Mahmood et al. showed that treatment with genistein and/or Eukarion (EUK)-207, which have antioxidant effects, can mitigate the release of inflammatory cytokines such as IL-1α, IL-1β, IL-6 and TNF-α, and reduce oxidative stress and activity of macrophages (Mahmood et al. 2011). BIO 300, a nanosuspension of genistein has shown similar results (Jackson et al. 2017). These studies show pivotal role of both free radical production and elevated activity of inflammatory mediators in development of radiation-induced pneumonitis.

In current study we aimed to detect possible mitigatory effects of two natural anti-inflammation and antioxidant agents. Curcumin demonstrated potent anti-inflammatory properties. However, it has low absorbance in intestine which may reduce its efficiency. In recent years, several studies have used other forms of curcumin with high absorbance. For instance, Nano-micelle curcumin is a low cost form of curcumin with easy absorbance. Results of this study showed that treatment with nano-micelle curcumin can strongly mitigate inflammation, infiltration of inflammatory cells, thrombosis, and vascular and alveolar injury. Mitigatory effect of nano-micelle curcumin may be mediated by suppression of inflammatory mediators such as NF-kB which in turn leads to reduced release of inflammatory cytokines. Additionally, curcumin has antioxidant properties and is able to reduce activity of pro-oxidant enzymes such as COX-2 and iNOS.

Alpha-tocopherol was another agent we used for mitigation of lung pneumonitis. Treatment with Alpha-tocopherol reduced the serum level of cytokines such as G-CSF, IL-10, IL-6, IL-12, and FLT3 in peripheral blood mononuclear cells (PBMCs) (Singh et al. 2014). Moreover, it was shown to mitigate intestinal injury following exposure to radiation through suppression of apoptosis and enhancement of cell proliferation (Singh et al. 2013). This study showed that post-exposure treatment with alpha-

tocopherol can mitigate infiltration of macrophages, lymphocytes and neutrophils. However, differences for alveolar and vascular was not significant. It is possible that longer follow-up or treatment with higher dose of alpha-tocopherol would be beneficial for a remarkable mitigation of radiation-induced lung pneumonitis.

CONCLUSION

This study showed that treatment with alpha-tocopherol or nano-micelle curcumin could mitigate radiation-induced pneumonitis in mice. However, alpha-tocopherol could not mitigate alveolar and vascular injury. Owing to the low toxicity of these agents, longer treatment or higher doses of alpha-tocopherol would be more effective. These findings may pave the way to mitigation of radiation toxicity after a radiation disaster such as nuclear explosion or radiological events.

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Availability of data and materials

All the relevant data and materials are presented in this article.

Authors' contributions

All authors were involved in this project.

Ethics approval

This study was approved by the Ethical Committee of the Baqiyatallah University of Medical Sciences on Animal Care.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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