Montelukast protects H9c2 cardiomyocytes against radiation-induced toxicity

Type

Research paper

Keywords

Oxidative stress, montelukast, Cardiomyocytes, leukotriene receptor antagonist, radiation-induced toxicity

Abstract

Introduction
Radiation-induced cardiotoxicity is a major concern following radiation treatment or involuntary radiation exposure. The normal functionality of cardiomyocytes becomes limited, which results in deleterious consequences, including an increase in the secretion of proinflammatory factors, such as HMGB1, and oxidative stress due to increased production of NOX4 and ROS. Radiation exposure results in disruption of the Bax/Bcl-2 ratio, increased expression of cytochrome c, and increased cleavage of caspase 3, thereby promoting cardiotoxicity and excessive apoptosis of cardiomyocytes. The cysLT1R antagonist montelukast is commonly used in the treatment of asthma, with recent studies showing potential for this drug in treating other diseases.

Material and methods
Rat H9c2 cardiomyocytes were used in all the vitro experiments. The MTT assay was used to determined the The Cell viability; DCFH-DA staning was used to measure the intracellular ROS; and TMRM method was used to determine the level of ΔΨm in H9c2 cells; All mRNA levels of gene were measured by real time-PCR

Results
In this study, we find that montelukast can mitigate the increase in oxidative stress and HMGB1 secretion induced by radiation, but also that montelukast exerts significant protective effects in terms of cell viability and apoptosis.

Conclusions
The authors of the present work hope to aid in the elucidation of the role of montelukast as a pretreatment for radiation and find safe and effective solutions to this common side effect of such a prevalent treatment in today’s society.

Title: Montelukast protects H9c2 cardiomyocytes against radiation-induced toxicity

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Abstract

Background: As we know, high levels of oxidation are also reflected by elevated levels of NADPH oxidase-4 (NOX-4) and malondialdehyde (MDA), which are known to contribute to many disease processes. And Radiation-induced cardiotoxicity is a major concern following radiation treatment or involuntary radiation exposure. The normal functionality of cardiomyocytes becomes limited, which results in deleterious consequences, including an increase in the secretion of proinflammatory factors, such as HMGB1, and oxidative stress due to increased production of NOX4 and ROS. Radiation exposure results in disruption of the Bax/Bcl-2 ratio, increased expression of cytochrome c, and increased cleavage of caspase 3, thereby promoting cardiotoxicity and excessive apoptosis of cardiomyocytes. The cysLT1R antagonist montelukast is commonly used in the treatment of asthma, with recent studies showing potential for this drug in treating other diseases. **Material and methods:** Rat H9c2 cardiomyocytes were used in all the vitro experiments. The MTT assay was used to determined the Cell viability; DCFH-DA staining was used to measure the intracellular ROS; and TMRM method was used to determine the level of ΔΨm in H9c2 cells; All mRNA levels of gene were measured by real time-PCR; and the protein levels of genes was determind by Western blots or ELISA. **Result:** In this study, we find that montelukast can mitigate the increase in oxidative stress and HMGB1 secretion induced by radiation, but also that montelukast exerts significant protective effects in terms of cell viability and apoptosis. **Conclusions:** The authors of the present work hope to aid in the elucidation of the role of montelukast as a pretreatment for radiation and find safe and effective solutions to this common side effect of such a prevalent treatment in today's society.

Keywords: Montelukast; radiation-induced toxicity; cardiomyocytes; oxidative stress; leukotriene receptor antagonist

1. Introduction

While radiation-based therapies for diseases such as cancer have provided considerable benefit overall, there remains great concern over the potential sequelae following exposure to radiation [1]. These include myocardial infarction, cardiovascular disease, and radiation-induced cardiotoxicity [2;3]. As a condition, radiation-induced cardiotoxicity is life-threatening and among the most difficult side effects of radiation therapy to treat [4]. Likely playing a major role in the development and sustainment of radiation-induced cardiotoxicity, accelerated cellular apoptosis is a paramount concern in the treatment of this condition [5]. Exposure to radiation has been shown to induce excessive production of reactive oxygen species (ROS), thereby inducing oxidative stress and further contributing to inflammation and cell death [6]. High levels of oxidation are also reflected by elevated levels of NADPH oxidase-4 (NOX-4) and malondialdehyde (MDA), which are known to contribute to many disease processes [7;8]. Some such downstream effects are the upregulation of apoptosis and inflammation. High-mobility group box 1 protein (HMGB1), a non-histone nuclear protein secreted by monocytes, plays a key role in inducing the inflammatory response via the production of proinflammatory cytokines. Additionally, HMGB1 has been found to increase the rate of apoptosis, which is further exacerbated in an oxidized state. This can lead to augmented cytotoxicity and in particular, can mediate the pathogenesis of radiation-induced cytotoxicity [9].

One of the key aspects in the prevention or treatment of radiation-induced cardiotoxicity is ameliorating the pathologic increase in cellular apoptosis. Bcl-2-associated X protein (Bax) is a member of the Bcl2 family and promotes apoptosis by creating channels in the outer mitochondrial membrane, thereby allowing translocation of mitochondrial proteins such as cytochrome c into the cytoplasm and initiating a proapoptotic signaling cascade. When expressed at healthy levels, this regulator helps to phase out old cells as part of the normal process of cell turnover. Bcl2, on the other hand, is an antiapoptotic gene which prevents excessive apoptosis and unnecessary cell death [10]. Thus, regulating the ratio of Bax/Bcl2 is an

important strategy for the treatment or prevention of radiation-induced cardiotoxicity.

Montelukast is a cysteinyl leukotriene type 1 receptor (cysLT1R) antagonist commonly used in the treatment of asthma and allergic rhinitis. Additionally, there is a growing body of research suggesting that montelukast possesses anti-inflammatory properties and may serve as a treatment for cardiovascular disease [11;12]. In the present study, we investigated the effects of montelukast on H9c2 cardiomyocytes to determine the potential of cysLT1R antagonism as a preventative treatment against radiation-induced cardiotoxicity.

2. Materials and methods

2.1 Cell culture and treatment

Rat H9c2 cardiomyocytes used in all experiments were purchased from the American Type Culture Collection (ATCC) (USA). The cells were cultivated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (1% penicillin/streptomycin). Briefly, the cells were pretreated with 5 or 10 μM montelukast for 6 h and then exposed to 8Gy radiation of 24 h.

2.2 Cell viability

To determine cell viability, we used a tetrazolium dye (MTT) assay. H9c2 cells were incubated in serum-free medium supplemented with 0.8 mg/ml MTT for 4 h. Then, dimethyl sulfide (DMSO) was added to the culture medium. The stabilized reaction mixture was then transferred into 96-well plates, and a microplate spectrophotometer system was to used to measure the absorbance at 560 nm.

Cytotoxicity was determined based on measurements of lactate dehydrogenase (LDH) leakage into the culture medium. Briefly, the culture medium was collected, and cell-free supernatants were collected. The fluorescent activity of LDH was assessed

using a commercially available kit from Thermo Fisher Scientific (USA). The results are expressed as percentages of the control values.

2.3 Reactive oxygen species (ROS) determination

To determine the level of intracellular ROS production, H9c2 cardiomyocytes were treated as described above and stained with 5 μM 2', 7'-Dichlorofluorescin diacetate (DCFH-DA) (Thermo Fisher Scientific, USA) in phenol-free red medium at 37 °C for 30 min. The cells were washed three times, and the fluorescent signals were visualized using a fluorescence microscope from Nikon (Japan). The fluorescence density of DCFH-DA was calculated using Image J software (NIH, USA). The results were quantified by defining regions of interest (ROI) and counting the number of positively stained cells in the ROI. Then, we assessed the integrated density value (IDV) of each ROI. The level of ROS=IDV/cell number.

2.4 Determination of ΔΨm

To determine the level of ΔΨm in H9c2 cells, we employed the tetramethylrhodamine methyl ester (TMRM) method. After the indicated treatment, the cells were incubated with 20 nmol/L TMRM for 1 h at RT. The cells were then washed 3 times, and an IBE2000 inverted fluorescence microscope (Zeiss, Germany) was used to detect the fluorescence signals. The resulting TMRM fluorescence density was calculated using Image J software (NIH, USA). To quantify our results, regions of interest (ROI) were defined, and the number of cells in each ROI were counted. The integrated density value (IDV) of the ROI was assessed. The level of ΔΨm = IDV/cell number.

2.5 Real-time polymerase chain reaction (PCR)

After the indicated treatment, total intracellular RNA was isolated from H9c2 cardiomyocytes using Qiazol reagent (Qiagen, USA). The concentration and quality of isolated RNA were determined using a NanoDrop ND1000 spectrophotometer. Then, 1 μg isolated RNA was used to produce cDNA via the reverse transcription

PCR (RT-PCR) method using an iScript RT-PCR kit from Bio-Rad (USA). To measure the expression of target genes, we performed real-time PCR analysis using a 7500 Real-Time PCR System (Applied Biosystems, USA) and a SYBR Green PCR Master Mix kit (Bio-Rad, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal housekeeping gene. The relative expression of the target gene was calculated using the $2^{-\Delta\Delta}$Ct method.

2.6 Enzyme-linked immunosorbent assay (ELISA)

Commercial ELISA kits were used to measure the protein expression levels of HMGB1. Briefly, the plates were coated with primary antibodies against the target proteins and incubated overnight at 4 °C. The plates were then washed 3 times with PBS solution and blocked with skim milk at RT for 1 h. Next, samples (50 µl) were added to each well of the ELISA plates and incubated overnight at 4 °C. The wells were then washed and incubated with primary antibodies for 1 h at RT, followed by incubation with corresponding secondary antibodies. The reaction was stopped using stop solution. Absorbance was recorded at 490 nm to determine the protein concentrations.

2.7 Westen blot analysis

The total cell lysate of H9c2 cardiomyocytes was prepared by lysing cells with RIPA buffer and protease and phosphatase inhibitor cocktail (Sigma-Aldrich, USA). A total of 20-40 µg cell lysates was loaded onto 4% PAGE gels and processed for 2 h to separate the proteins according to size. The gel was then transferred to a PVDF membrane. Next, the blots were blocked with 5% non-fat milk and incubated with specific primary antibodies and corresponding secondary antibody. The immunoblots were visualized using a Pierce™ SuperSignal West Pico Plus system.

2.8 Statistical analysis

The experimental results are expressed as means ± standard error of the mean (S.E.M).

Comparisons between groups were performed using one-way ANOVA and analyzed by SPSS software (Version 20), followed by the Bonferroni's test. A value of $P < 0.05$ was considered a statistically significant difference.

3. Results

3.1 Montelukast rescues cardiomyocytes from radiation-induced cell death

We found that upon exposure to 8Gy radiation alone, cell viability decreased by 43%. This decrease was mitigated by pretreatment of H9c2 cells with 5 and 10 μM montelukast for 6 h in a dose-dependent manner. Upon treatment with 5 μM montelukast, cell viability was decreased by 17%, while the 10 μM dose resulted in a decrease of only 5%, thereby demonstrating a significant protective effect on cell viability (Figure 1A). Furthermore, we studied the effect of montelukast on levels of LDH release. Upon exposure to radiation, the release of LDH increased by 20.3% from basal levels. Montelukast pretreatment downregulated this increase in LDH release to only 7.9% and 4.8% in a dose-dependent manner (Figure 1B).

3.2 Montelukast ameliorates radiation-induced oxidative stress and reduced ΔΨm

Levels of ROS and MDA play a key role in oxidative stress, which compromises cell viability and promotes inflammation. When exposed to radiation alone, the level of intracellular ROS in H9c2 cells increased roughly 3.4-fold from basal levels. However, the two doses of montelukast were able to reduce the level of ROS to only approximately 2.5- and 1.6-fold, respectively (Figure 2A). The initial radiation-induced upregulation of MDA was nearly 3-fold higher than basal levels, but the two doses of montelukast reduced the level of MDA to only 2.2- and 1.5-fold, respectively (Figure 2B). Next, we tested the effect of montelukast on radiation-induced reduced levels of mitochondrial membrane potential (ΔΨm). The initial reduction in ΔΨm was 55%, while pretreatment with montelukast rescued this

reduction to only 28% and 6% in a dose-dependent manner (Figure 3).

3.3 Montelukast reduces the expression of NOX-4 and HMGB1

Our findings demonstrate that NOX-4 mRNA was significantly upregulated upon exposure to radiation, increasing nearly 4-fold. Montelukast reduced this increase to 2.5- and 1.6-fold, thereby demonstrating a dose-dependent inhibitory effect of cysLT1R antagonism on NOX-4 mRNA expression (Figure 4A). We also found that at the protein level, radiation induced an increase in NOX-4 expression of 3.3-fold, which was reduced to 2.1- and 1.4-fold by the respective doses of montelukast (Figure 4B). Next, we investigated the effects of montelukast on the expression of HMGB1. Here, we found that montelukast significantly ameliorated radiation-induced overexpression of HMGB1. Upon exposure to radiation alone, the expression of HMGB1 was increased from 354.5 to 1322.8 pg/ml. Meanwhile, the two doses of montelukast reduced the concentration of HMGB1 protein to 877.5 and 654.9 pg/ml (Figure 5).

3.4 Montelukast prevents radiation-induced apoptosis

To investigate the effects of montelukast on radiation-induced cellular apoptosis, we first determined that the level of apoptosis under normal conditions was roughly 8% but was upregulated to approximately 41.3% upon exposure to radiation. However, pretreatment with the two doses of montelukast reduced the level of radiation-induced apoptosis to only 27.9% and 15.6%, respectively (Figure 6).

3.5 Montelukast improves the Bax/Bcl-2 ratio

The Bax/Bcl-2 ratio is important in maintaining healthy organ function and cell turnover due to its regulatory role in the apoptotic process. Here, we found that radiation-induced a 4.5-fold increase in Bax as compared to Bcl2. However, montelukast pretreatment reduced this increase to only 2.5- and 1.6-fold (Figure 7).

3.6 Montelukast prevents cytochrome C release and caspase 3 cleavage

Finally, we measured the release of cytochrome c and cleavage of caspase 3 in radiated cardiomyocytes. We found that exposure to radiation increased the release of cytochrome c 3.8-fold, which was reduced to only 2.4- and 1.6-fold by pretreatment with montelukast in a dose-dependent manner (Figure 8A). Cleavage of caspase 3 was increased roughly 4.5-fold upon exposure to radiation. However pretreatment with the two doses of montelukast reduced the level of cleaved caspase 3 to only 2.5- and 1.3-fold in a dose-dependent manner (Figure 8B).

4. Discussion

The development of cardiotoxicity due to radiation is an inevitable side effect of treatments such as radiation chemotherapy for cancer. While vital in the alleviation of certain diseases for which a safer treatment option does not yet exist, the use of radiation therapy can cause irreversible damage to the heart [13]. Our results demonstrate a major cell death-inducing mechanism of radiation which caused an initial downregulation of cell viability by nearly half. Furthermore, the release of LDH was monitored due to its status as a marker of cellular necrosis [14]; the level of LDH was found to be significantly upregulated in radiated H9c2 cells in the present study, which is in concurrence with previous research [15]. Here, we found that the cysLT1R antagonist montelukast demonstrated an acute ability to ameliorate radiation-induced cell death in a dose-dependent manner. Previously shown to be involved in the process of apoptosis, the radiation-induced downregulation of $\Delta\Psi m$ has significant consequences on cell health and function [16;17]. Previous research has shown a potential ability of cysLT1R antagonism to ameliorate the loss of $\Delta\Psi m$ induced by ischemic injury [18]. Here, we found that montelukast significantly rescued radiation-induced loss of $\Delta\Psi m$ in cardiomyocytes. This is the first study to our knowledge to demonstrate this ability of montelukast.

Oxidative stress induced by overproduction of ROS is a key component in

radiation-induced cytotoxicity [19]. Oxidative stress is characterized by an imbalance in the ratio of oxidants to antioxidants and has been shown to play a significant role in radiation-induced cell death [20]. Numerous studies have demonstrated the antioxidant power of montelukast in various cell types [21-23], but to our knowledge, the present study is the first to explore the potential of this drug in treating radiation-induced cardiotoxicity. We observed a significant reduction in the level of ROS in radiated cardiomyocytes after pretreatment with montelukast. Known for playing a key role in guiding the production of ROS, NOX-4 is another crucial player in the pathogenesis of radiation-induced cardiotoxicity [24]. Downregulation of NOX-4 has been previously found to reduce ROS production and importantly, to decrease the rate of radiation-induced cell death [25]. Taken in conjunction with the present research which demonstrated an acute ability of montelukast to lower NOX-4 expression in radiated cells, these findings suggest a potent antioxidant capacity of montelukast, which is of value in the treatment and prevention of radiation-induced cardiotoxicity.

HMGB1, an inflammatory response-inducing catalyst, has been implicated in the pathogenesis of radiation-induced cardiotoxicity [26]. HMGB1 is vital in mediating the cellular stress response, but in certain conditions such as during radiation exposure, HMGB1 can be secreted extracellularly. Upregulated release of HMGB1 results in a state of significant inflammation, which perpetuates radiation-induced cellular damage [27]. So far, there has been little research on the role of cysLT1R in HMGB1 secretion. In the present study, pretreatment with montelukast was found to substantially reduce levels of HMGB1 secretion in human H9c2 cardiomyocytes, demonstrating a potential anti-inflammatory property of cysLT1R antagonism. Apoptosis has long been recognized as a result of radiation exposure. An extensive body of work exists linking the two and theorizing the exact processes through which this relationship occurs [28;29]. Recent research has highlighted a prominent role of ROS overproduction in radiation-induced apoptosis, but the exact mechanisms are not yet fully understood [30]. Additionally, HMGB1 has been shown to be secreted by

apoptotic cells [31]. As mentioned above, ROS overproduction and secretion of HMGB1 were successfully inhibited by montelukast pretreatment. We further demonstrated the inhibitory effect of montelukast on cell apoptosis through flow cytometry and found that montelukast significantly inhibited radiation-induced apoptosis.

The ratio of Bax/Bcl-2 has long been utilized as a predictive marker for response to radiotherapy in various types of cancer including breast and prostate [32;33] However, there is little research regarding the relationship between cysLT1R and Bax/Bcl2 and no studies to our knowledge investigating the effects of montelukast on Bax/Bcl2 ratio. Here, we found that pretreatment with montelukast rescued the increase in Bax induced by radiation, thereby indicating the involvement of cysLT1R in regulating apoptosis in cardiomyocytes. These findings are supported by our results showing that montelukast significantly reduced the radiation-induced release of cytochrome c from mitochondria and cleavage of caspase 3 induced by radiation, both markers of apoptosis [34].

Other mechanisms may also be involved in radiation-induced cardiotoxicity. The serum level of endocan has been identified as a risk factor for cardiotoxicity [35, 36]. The production of nitric oxide (NO) (both inducible NO and endothelial NO) is another risk factor associated with radiation-induced lethal injury [37], and a recent study shows that the NO pathway plays a key role in ischemia/reperfusion-induced injury [38]. Although we did not pursue these molecules in our study, it is possible that the induction of endocan and NO could play a role in radiation-induced cardiotoxicity. Future investigation will provide a complete picture of the underlying mechanism.

Some limitations of our study have to be addressed. Although embryonic rat cardiomyocyte-derived H9c2 cells have been used as a convenient *in vitro* model for drug testing and the investigation of cardiac hypertrophy [39,40]. However, these cells do not precisely replicate the structural and functional physiology of primary isolated cardiomyocytes. For example, H9c2 cells exhibit differentiation potential towards adult skeletal phenotypes, and they do not spontaneously beat in culture and show different

responses to some stimuli when compared to other neonatal cardiomyocytes [41]. Therefore, tests using H9c2 cell culture may not perfectly simulate physiological conditions *in vivo*. Animal models of whole thorax, whole heart, and partial heart irradiation have been established to study cardiac radiation toxicity *in vivo* [42]. Future use of animal models guided by advanced imaging technology will allow more accurate evaluation of the effect of montelukast on radiation-induced cardiotoxicity.

The possible cardioprotective effect of anti-oxidative agents have been reported before. For example, a recent study reported that the anti-oxidative agent carvedilol possesses a protective effect against cadmium (Cd)-induced cardiotoxicity by regulating endothelial nitric oxide synthase (eNOS) production and HO-1/Nrf2 signaling [43]. However, the relation between antioxidant status and overall survival in cancer patients remains controversial. Clinical studies have shown that indicators of antioxidant parameters in patients with renal cell carcinoma are not associated with overall survival [44]. An important focus of radiation therapy is the sensitization of DNA damage machinery to kill tumor cells, but the action of radiation also involves the formation of ROS and damage to antioxidants in tissues [45]. A recent report using a cataract animal model suggests that widely consumed caffeine exerts a therapeutic effect by inhibiting ROS production [46]. Based on the findings of this study, we speculate that the beneficial role of anti-oxidative agents in radiation therapy might have preventive and therapeutic implications.

In conclusion, our findings demonstrate a potent ability of montelukast to prevent radiation-induced cardiotoxicity by rescuing cell viability, suppressing oxidative stress and inflammation, and reducing apoptosis. These effects are the result of reduced production of ROS via suppression of NOX-4 and reduced HMGB1 secretion. Importantly, we also demonstrate the ability of montelukast to improve the Bax/Bcl2 ratio in radiated cardiomyocytes. Together, our findings demonstrate the potential of montelukast as a preventative treatment against radiation-induced cardiotoxicity. However, further research using animal models is required to gain a better understanding of the mechanisms involved.

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Legends

Figure 1. Montelukast protected H9c2 cardiomyocytes against radiation-induced cell death. H9c2 cells were pretreated with montelukast (5, 10 μM) for 6 h, followed by exposure to radiation (8Gy). (A). Cell viability was measured by MTT assay; (B). Release of LDH was assayed (***, P<0.001, ****, P<0.0001 vs. control group; ##, P<0.01, ###, P<0.001 vs. radiation group; $, P<0.05 vs. radiation+5 μM montelukast).

Figure 2. Montelukast protected H9c2 cardiomyocytes against radiation-induced oxidative stress. H9c2 cells were pretreated with montelukast (5, 10 μM) for 6 h, followed by exposure to radiation (8Gy). (A). Intracellular ROS was measured byDCFH-DA; (B). Intracellular level of malondialdehyde (MDA) (****, P<0.0001 vs. control group; ##, P<0.01, vs. radiation group; $$, P<0.01 vs. radiation+5 μM montelukast).

Figure 3. Montelukast protected H9c2 cardiomyocytes against radiation-induced

reduced mitochondrial membrane potential (ΔΨm). H9c2 cells were pretreated with Montelukast (5, 10 μM) for 6 h, followed by exposure to radiation (8Gy). Intracellular ΔΨm was assayed by tetramethylrhodamine methyl ester (TMRM) staining (***, P<0.001, ##, P<0.01 vs. radiation group; $, P<0.05 vs. radiation+5 μM montelukast).

Figure 4. Montelukast protected H9c2 cardiomyocytes against radiation-induced expression of NADPH oxidase 4 (NOX-4). H9c2 cells were pretreated with Montelukast (5, 10 μM) for 6 h, followed by exposure to radiation (8Gy). (A). mRNA of NOX-4; (B). Protein of NOX-4 (****, P<0.0001 vs. control group; ##, P<0.01, vs. radiation group; $$, P<0.01 vs. radiation+5 μM montelukast).

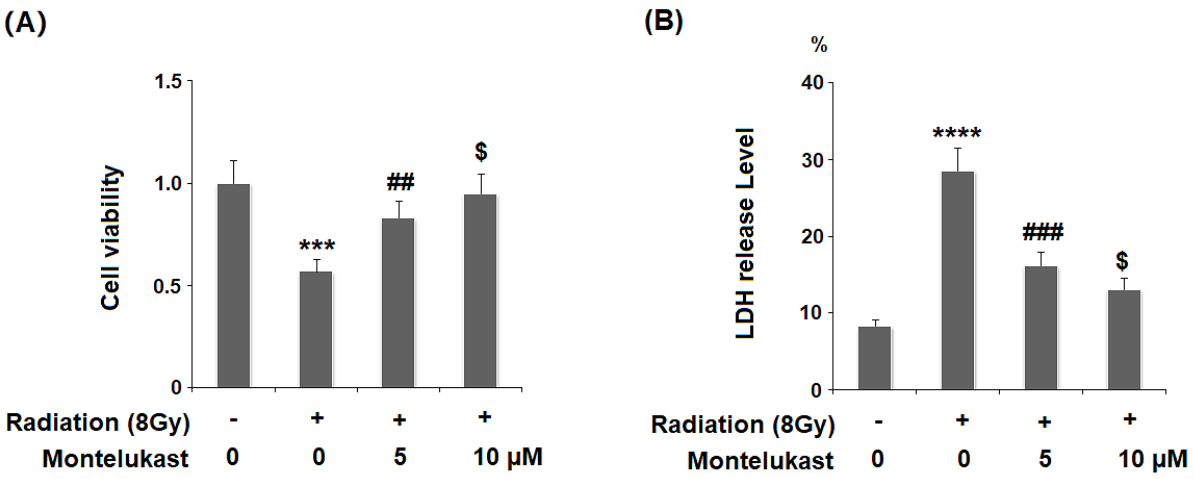
Figure 5. Montelukast protected H9c2 cardiomyocytes against radiation-induced secretions of high-mobility group box-1 (HMGB1). H9c2 cells were pretreated with Montelukast (5, 10 μM) for 6 h, followed by exposure to radiation (8Gy). Secretions of HMGB1 was measured by ELISA assay (****, P<0.0001 vs. control group; ###, P<0.001 vs. radiation group; $, P<0.05 vs. radiation+5 μM montelukast).

Figure 6. Montelukast protected H9c2 cardiomyocytes against radiation-induced apoptosis. H9c2 cells were pretreated with montelukast (5, 10 μM) for 6 h, followed by exposure to radiation (8Gy). Cell apoptosis was measured by flow cytometry (****, P<0.0001 vs. control group; ##, P<0.01, vs. radiation group; $$, P<0.01 vs. radiation+5 μM montelukast).

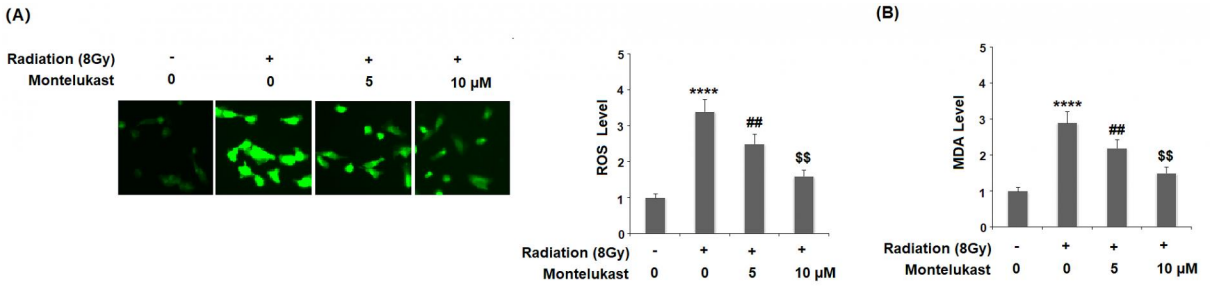
Figure 7. Montelukast protected H9c2 cardiomyocytes against radiation-induced change in the ratio of Bax/Bcl-2. H9c2 cells were pretreated with montelukast (5, 10 μM) for 6 h, followed by exposure to radiation (8Gy). (A). Protein expression of Bax and Bcl-2 as measured by western blot analysis; (B). Statistical analysis of Bax/Bcl-2 (****, P<0.0001 vs. control group; ###, P<0.001, vs. radiation group; $, P<0.05 vs. radiation+5 μM montelukast).

Figure 8. Montelukast protected H9c2 cardiomyocytes against radiation-induced release of cytochrome C and the cleavage of caspase 3. H9c2 cells were pretreated

with montelukast (5, 10 μM) for 6 h, followed by exposure to radiation (8Gy). (A). The release of cytochrome C from mitochondria to cytosol; (B). Levels of cleaved caspase 3 (****, P<0.0001 vs. control group; ###, P<0.001, vs. radiation group; $, P<0.05 vs. radiation+5 μM montelukast).



Montelukast protected H9c2 cardiomyocytes against radiation-induced cell death.



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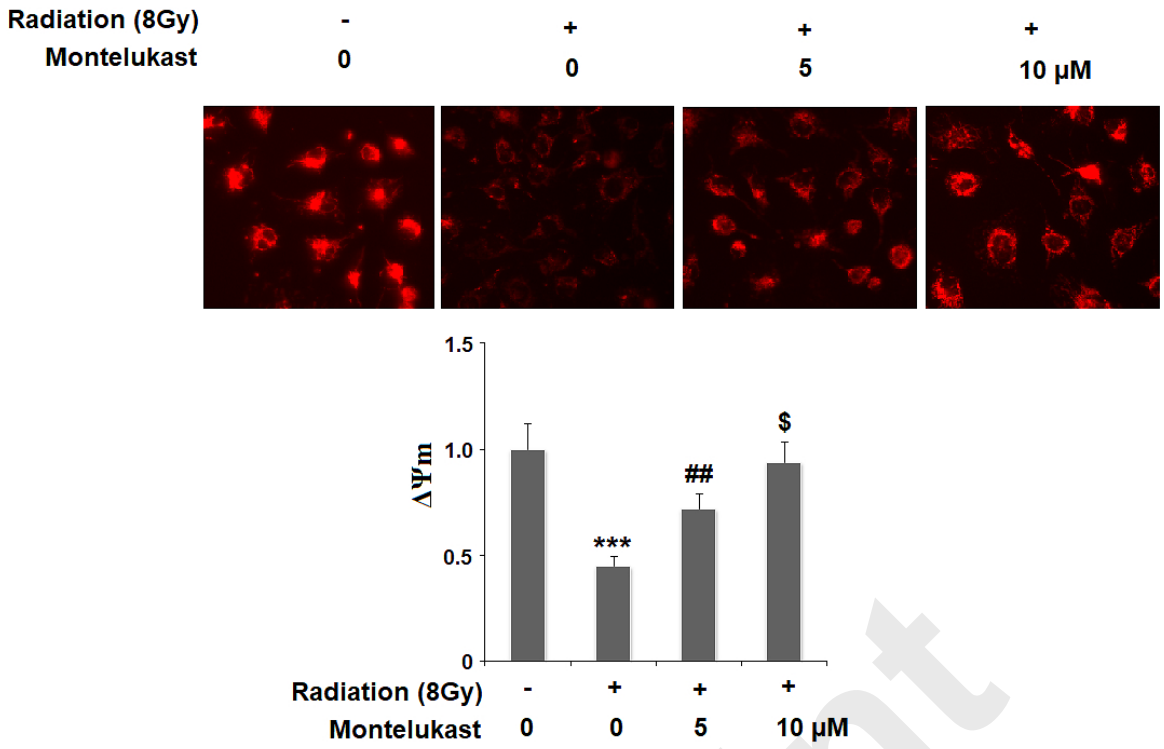
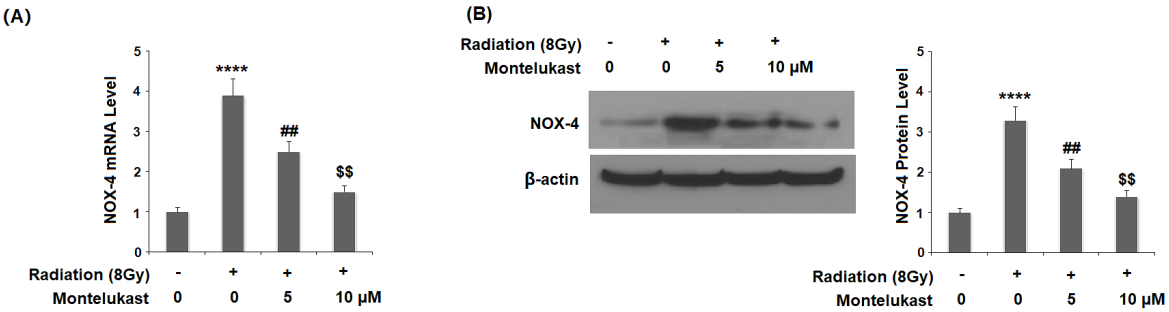
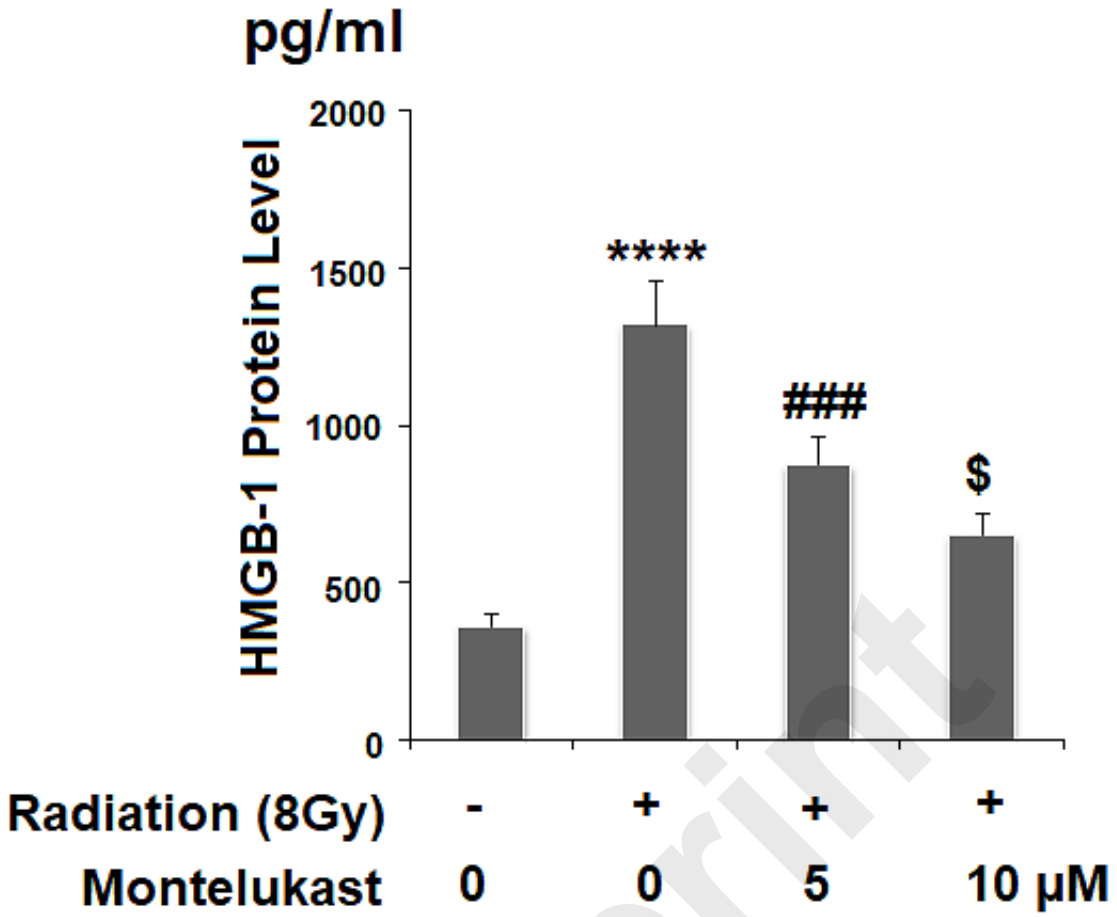


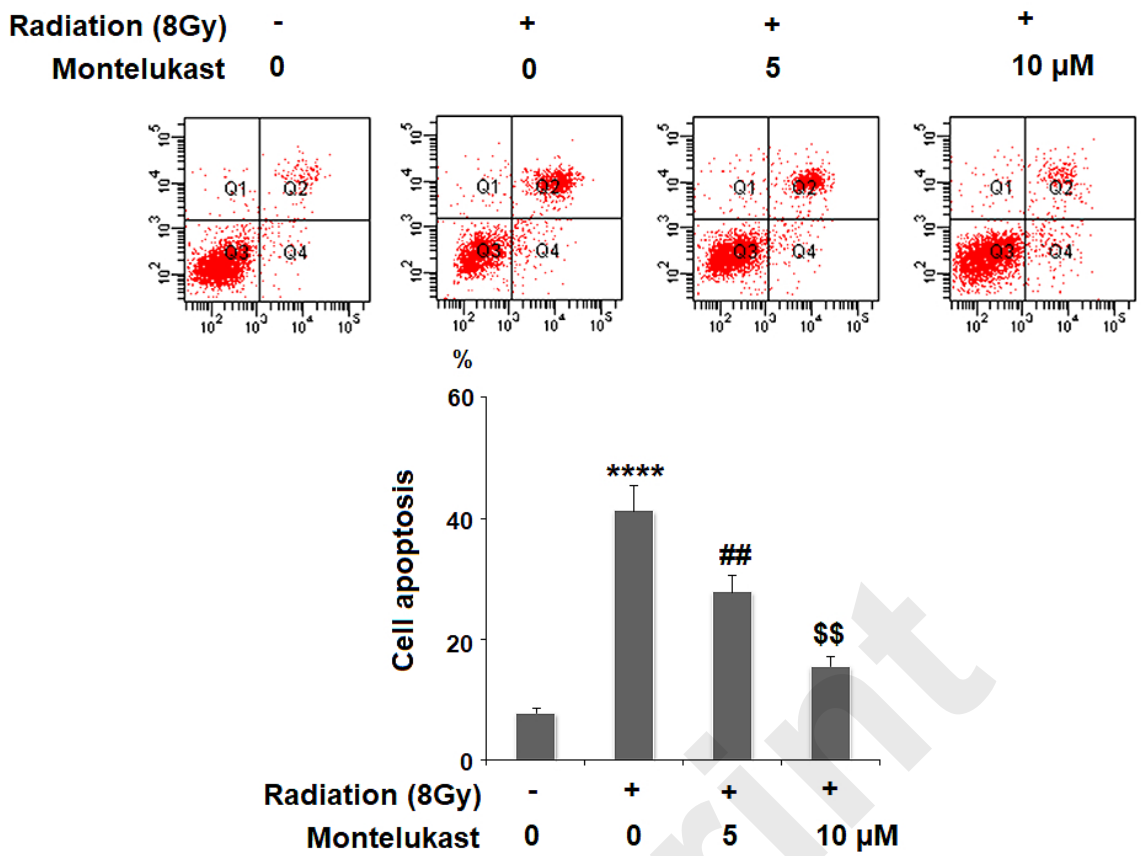
Montelukast protected H9c2 cardiomyocytes against radiation-induced reduced mitochondrial membrane potential (ΔΨm).



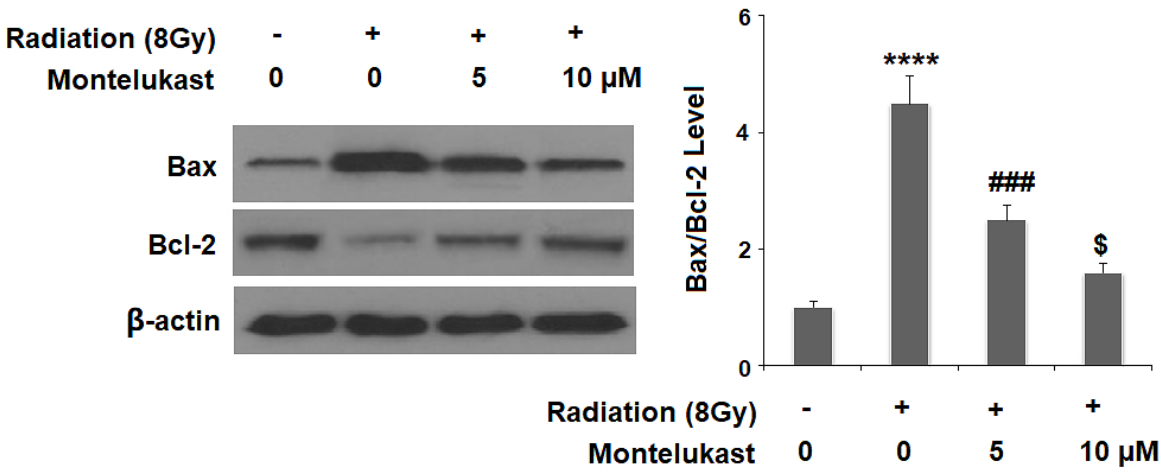
Montelukast protected H9c2 cardiomyocytes against radiation-induced expression of NADPH oxidase 4 (NOX-4).



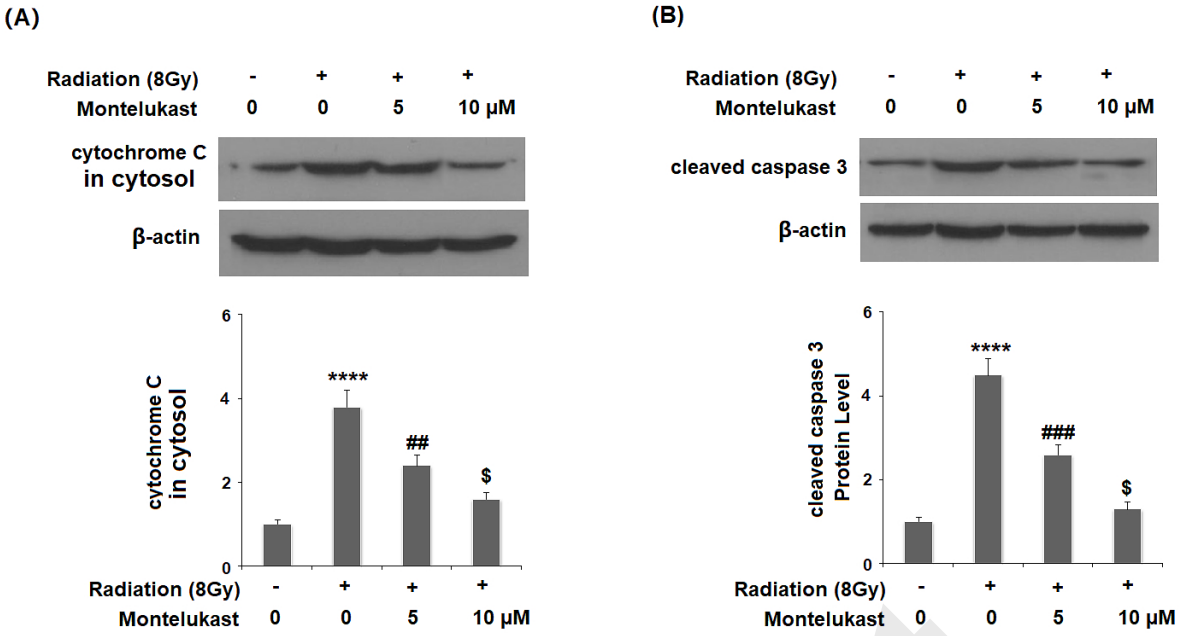
Montelukast protected H9c2 cardiomyocytes against radiation-induced secretions of high-mobility group box-1 (HMGB1).



Montelukast protected H9c2 cardiomyocytes against radiation-induced apoptosis.



Montelukast protected H9c2 cardiomyocytes against radiation-induced change in the ratio of Bax/Bcl-2.



Montelukast protected H9c2 cardiomyocytes against radiation-induced release of cytochrome C and the cleavage of caspase 3.