The Role of Protein Kinase Activators and ZFN in Reducing the Harmful Effects of Total Body Irradiation

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Research note

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Abstract

Objective

AIDS patients are predisposed to develop certain tumors. Radiation therapy is one of the main treatments for cancer. However, exposure to ionizing radiation leads to defects in the lymphatic system. The cells with the highest sensitivity to radiation are the T lymphocytes. We aim to investigate the effect of irradiation on the ratio of CD4 to CD8, and to oppose this effect by using Bryostatin (B) and ZFN and ART (Anti-Retroviral Therapy).

So Balb/C mice aged 6-8 weeks were whole-body irradiated with 1.5 Gy of γ-rays, was then mice were treated with ZFN, Bryostatin intraperitoneally. And orally Antiviral treatment. At the end of the study, CD4/CD8 was measured by flow cytometry.

Results

Irradiation caused significant pathological changes that led to a clear reversal of CD4/CD8 ratio significantly within the normal radiating group. there are no significant differences in CD4/CD8 ratio between all groups treated with ZFN+ X, Z+ B+ X, ART+ X and normal group.

So our study suggests the effect of Bryostatin in protecting living organs that were exposed to irradiation in mice. ZFN cuts off both BAX and BAK, and thus decreases apoptotic proteins, reducing cell death.

Introduction

AIDS patients have a high percentage of special tumors, especially Kaposi sarcoma (KS), non-Hodgkin lymphomas and cervical cancer in women. The causes for the occurrence of poly human tumors include the decrease in the number of T cells, that are responsible for immunity, the irregular functioning of both B cells and monocytes [1].

Radiation therapy is one of the main treatments for cancer along with surgery and chemotherapy. Ionizing radiation is used to destroy cancer cells. Radiation therapy has been used for more than 100 years to treat nearly all solid tumors such as skin, brain, breast, prostate and cervical cancers, and it can also be used to treat leukemia, lymphoma and glioma [2].

However, Ionizing radiation also causes natural tissue injury, leading to cell death.[2].

Exposure to ionizing radiation leads to defects in the lymphatic system and blood components, through a complex chain of events, known as blood-forming cell syndrome, which can lead to septicemia and death [3]. The most sensitive to irradiation, are the T lymphocytes, which are CD4 and CD8 [4]. In this study, we investigate the effect of irradiation on the ratio of CD4/CD8.

Some drugs can oppose the effect of radiation, like antioxidant drugs, but there is some other groups like protein kinase activators, e.g. Bryostatin (B) and ZFN (Z) which are used as antiretroviral drugs through gene, were investigated for the possibility of opposing the damage caused by irradiation, since they are already prescribed for AIDS patient.

Methods

Five groups of Balb/C mice aged 6-8 weeks [5] with weights ranging from 25-30 g were used. The mice were obtained from the animal house of Syrian scientific research (Damascus, Syria). Each group contained 10 mice. The animals were maintained under natural condition (~14-hour light and 10-hour dark full-spectrum light/dark cycles and at 20–25°C with 40–70% relative humidity).

All animals received humane care in compliance with National Research Council criteria outlined in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources – Commission on Life Sciences
1996), [6] ,and with authorization of the Institutional Animal Care and Use Committee (Faculty of pharmacy).

Animals groups:

The mice were divided into 5 groups; each group contained 10 mice, each group divided into two cages, the cage contained 10 mice: normal (control group), normal+ irradiation (X), group treated with ZFN (X+ ZFN), group treated with ZFN+ Bryostatin (X+ZFN+BRY), and finally a group treated with ART.

Irradiation:

A Gamma Theratron-80 Irradiator with Cobalt-60 source was used (Theratron 80 from Atomic Energy Canada Limited, Canada). 80 balb/c mice of age 6-8 weeks were administered to a total body irradiation (TBI) of 1.5 Gy at a dose rate of 0.85 Gy. min^{-1}. Each 10 mice were putted in plastic rounded box with 30 cm diameter and 5 cm high to prevent crowded.

Treated groups:

ZFN (cusabio, china) was injected (3,100 ng,) intraperitoneally, 2-4 hours after exposing mice to irradiation [7].

, Bryostatin (sigma, Germany) 40 µg/kg injected intraperitoneally [8].and Antiviral treatment was continue dorally for seven weeks [9], tenofovir 4.5 mg and emtricitabine 3 mg, from the NRTI group, and from the NNRTIs group, efavirenz was used at a dose of 18 mg per day [10].

At the end of the study, blood samples were withdrawn from the retro-orbital mouse eye for tests of flow cytometry. And all animals were killed by anesthesia of over dose of ether.

Flow cytometry analysis

A sample of blood is withdrawn (1-2.5 ml), then 300 µl is transferred from the blood sample to a new tube then phosphate-buffered saline (PBS) solution is added, then the blood thinning, and the float is thrown.

5 µl of rat anti mouse solution CD16/CD32 was added to the washed blood sample and brood on ice for 15 minutes.

In the meantime, a mixture of monoclonal antibodies combined with fluorinated pigments is added new tubes (to each tube) in appropriate quantities according to the publication of the anti-body production company, In addition to other three other: in the first tube is added a mixture of immunoglobulins combined with The same fluorinated dye that is marked by the antibodies and this tube is called the control sample for immune coloring or isotype control, and to the second tube is added 100 µl of washing solution and called the sample controlling self-fluorine, and To The third tube is added 100 µl of PBS solution contained the propidium iodide) PI(, and called the viability control sample. to each tube is added 50 microliters of washed blood, and after brooding on the ice in a dark place, the lysis solution of the red blood cells is added, then the tubes were tauning and float, then the cells were washed with PBS solution with the tauning and floating, finally the cells in each tube are attached in 500 microliters of PBS solution contained on paraformed aldehyde at a concentration of 1%.

The samples are then analyzed by the flow cell meter as instructed by the manufacturer, record measurements of 10,000 cells, and then analyze the data using the data analysis software.

Statistical analysis:

Data were analyzed by applying One way ANOV followed by the Tukey Multiple Comparison, with 95% confidence level. The P-values <0.05 were considered to be significant.

Results

The results (table 1) shows that the exposure of mice to radiation led to significant pathological changes, that lead to a clear reversal of CD4/CD8 ratio within the normal radiating control group (NORMAL X) in a statistically significant manner (P<0.001) compared to normal, Figure 1.

Table 1
CD4/CD8 ratio among treated and radiation-exposed groups

NORMAL+X	ZFN+X	Z+B+X	ART+X	
0.5	1.4	1.8	1.5	
0.7	1.3	1.6	1.7	
0.4	1.4	0.9	1.3	
0.6	0.7	1.5	1.5	
1.1	0.9	0.9	1.4	
1.4	1.6	1.7	1.1	
0.5	1.5	1.5	1.5	
1.2	1.3	1.6	1.4	
0.9	1.3	0.9	1	
1.7	1.6	1.4	1.4	
0.9	1.3	1.38	1.38	Average
0.437162568	0.290593	0.348967	0.204396	SD

The statistical study also shows that there are no significant differences in CD4/CD8 ratio between all groups treated with ZFN+ X, Z+ B+ X, ART+ X and the control group.

The study shows that ZFN, ART and Bryostatin can counteract the pathological changes of irradiation, where CD4/CD8 ratio in the treated and irradiated groups, ART+ X and Z +B +X, was statistically significantly higher when compared to the control irradiated group at the level of indication ($P<0.05$).

Discussion

Our study showed a clear reversal in CD4/CD8 ratio after exposure to radiation, compared with the control group, and this was agreed with several studies, which indicated that irradiation can destroy the immune system to a specific degree through a lack of peripheral lymphocyte count, including CD4 and CD8. [11].

However, these studies differed in the evaluation of radiation sensitivity and were as follows:

1- Some studies did not indicate any difference, in CD4:CD8 ratio as a result of radiation exposure [12].

2- Other studies indicated a clear decrease in CD8 count, and thus a clear increase in CD4:CD8 rate.[13]

Where CD4 is more resistant to radiation at 2-Gray dose than CD8, but CD8's return to normal levels is faster than CD4's [14]. This theory proved to be striking, and after a time of exposure to nuclear bomb radiation, there was a clear variability in the cell count, with a normal CD4 cell count and a decrease in CD8 cell count in the exposed organisms [15] Plus, with the exception of only one report, the CD4 cell count increased in people exposed to radiation in the Chernobyl tragedy [15].

3- However, other studies have suggested that CD4 is more sensitive than CD8, and there was a decrease in CD4:CD8 ratio [16]. This was agreed with our research (Table 1) where a clear reversal in CD4/CD8 ratio within the normal-radiated control group (NORMAL+ X) was observed ($P<0.001$) compared to the normal control group.

The reason for the decrease in CD4 cells is that CD4 lymphocytes show elevated levels of apoptosis in response to radiation, associated with high levels of Ser46 phosphorylation on the protein p53 ,which cause apoptosis [17].

After that , the cells recover from radiation, where previous studies have shown that Granulocyte colony-stimulating factor (G-CSF) promotes hematological blood healing, where it enhances the differentiation and reproduction of myeloid progenitor cells [18]. As G-CSF is activated, stem cells migrate to the thymus and may induce their reproduction and differentiation within the thymus [18].

The thymus is the primary site of T-cell development, but this organ is very sensitive to radiation. Radio damage is transient, and regeneration is rapid and clear, so CD8/CD4 returns to normal levels within 14 days. And G-CSF synergizes with cytokines like IL-1 , IL-3 ,IL-6, develop the thymus cells [18].

However, our research did not indicate a recovery in CD4:CD8 frequency, which may be due to bone marrow injury as well as thymus injury, because many researches have indicated that the action and size of the thymus decreases within 6 months when animals exposed to radiation at a dose of 2-5 Gray [19]. It is worth noting that the re-synthesis depends not only on the proliferation of precursors resistant to radiation within the thymus, but also from the mobilization of stem cells from the bone marrow to the thymus, where they differentiate to become mature T cells [18].

Our study showed the ability of Bry+ ZFN and antivirals to treat radiation damage and return CD4/CD8 to normal levels. Several studies have indicated the ability of bryostatin to protect living organs from irradiation in irradiation-exposed mice. The mechanism by which protein kinase activators enhance radiation protection is that the activation of accessory cells by bryostatin causes the release of growth factors, such as G-CSF, IL-1 and IL-6 which have a clear role in radiation protection. Bryostatin may modify the action of GM-CSF Granulocyte-macrophage colony-stimulating factor [20].

Activating of Pk-C signals by bryostatin may also contribute to the expression of certain oncogenes, such as c-jun tumor genes, which interfere with the cell's protection from radiation harm [20]. Bryostatin enhances the development of advanced stem cells in mouse bone marrow, i.e. it enhances repairs in bone marrow after exposure to radiation [21].

However, other studies have indicated that protein kinase activators have a role in the apoptosis induced by irradiation through ATM; it inhibits the production of ceramide, which prevents the cell from getting into apoptosis, where PKCa causes a reregulation or negative regulation of ATM proteins. The negative regulation forces cells to enter the stage of apoptosis but does not cause real apoptosis. In addition to passive regulation of ATM, the apoptosis needs radiation [22].

The role of ZFN, Exposure to radiation causes an increase in p53, resulting from the break-up of DNA. P53 then phosphorylates many of its target genes, including BAX and PUMA, which are sufficient to cause cell death [23]. Here the role of ZFN is clear, as it cuts off both BAX and BAK, and thus decreases in apoptotic proteins and reduces cell death [24].

Antiviral Drugs, It has been previously indicated that antiviral drugs cause the depletion of mitochondrial DNA, subsequently depleting DHODH, reducing pyrimidine concentrations and decreasing lymphatic reproduction [25]. So The decrease in lymphatic production includes both CD4 and CD8 i.e. decrease in the entire proportion, this is observed in clinical trials in which patients took didanosine, which is from (NRTIs), and this is what our results show [25].

Conclusion

Our study showed the ability of Bry+ ZFN and antivirals to treat radiation damage and return CD4/CD8 to normal levels. Because bryostatin activtaes accessory cells causes the release of growth factors, such as G-CSF, IL-1 and IL-6 which have a clear role in radiation protection. bryostatin may also contribute to the expression of c-jun tumor genes, which interfere with the cell's protection from radiation harm. Bryostatin enhances the development of advanced stem cells in mouse bone marrow, i.e. it enhances repairs in bone marrow after exposure to radiation, But the role of ZFN is clear, as it cuts off both BAX and BAK, and thus decreases in apoptotic proteins and reduces cell death. And Antiviral Drugs:cause the depletion of mitochondrial DNA, subsequently depleting DHODH, reducing pyrimidine concentrations and decreasing lymphatic reproduction.

List Of Abbreviations

Bry: Bryostatin, X:irradiation, ZFN: Zinc Finger Nuclease, ART: Anti Reroviral Therapy.

Declarations

Ethics approval and consent to participate

All animals received humane care in compliance with National Research Council criteria outlined in the Guide for the Care and Use of Laboratory Animals [6] ,and with authorization of the Institutional Animal Care and Use Committee.

Availability of data and material

Not applicable

Consent to publish

Not applicable

Competing interest

The authors declare that they have no competing interest.

Authors' Contributions

All authors contributed equally, by providing scientific orientation and reviewing the manuscript. All authors read and approved the final manuscript

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Figures

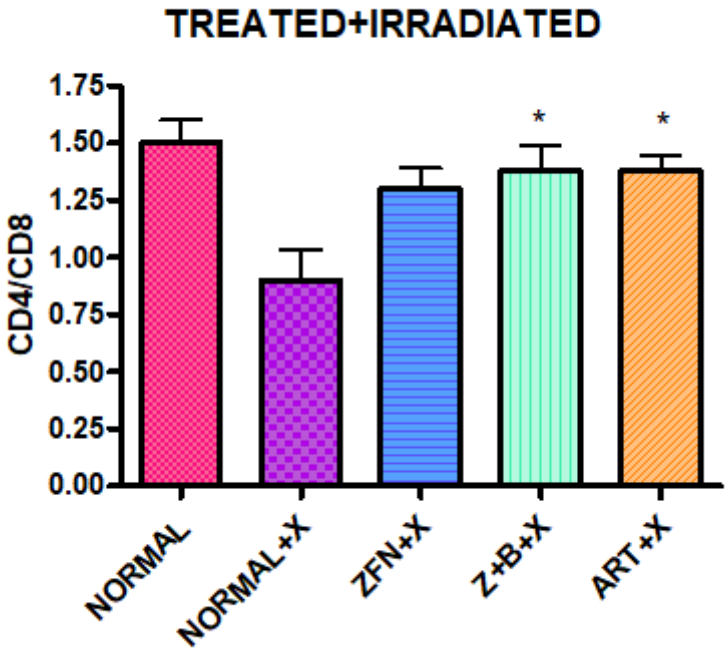

Figure 1

A graph showing the effect of irradiation and treatment with ZFN, ZFN +Bryostatin and antiviral (ART) on CD4/CD8 cell ratios compared to normal and irradiate control group.

Supplementary Files

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