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Activation Role of Low Dose Ionizing Radiation on Human Lymphocytes Response

S.A. Montaser[#], G.R. Abdel Hamid and A.R. Aboghadeer
Radiation Biology Department, National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt.

LOW dose ionizing radiation can imitate the role of deteriorate antigen or attenuated microbes as it activates the first defence line of immune response, the step which when lost, cancer cells can find suitable environmental condition for growth propagation. Human whole blood samples were irradiated *in vitro* at doses (0.25, 0.5, 0.75 and 1Gy) of ionizing radiation and then kept at 37°C for 1h before measuring glucose concentrations which reflect the maximum activation of immune cells. Then, immunological parameters such as interlukine-1β (IL-1β), interlukine-2 (IL-2), transforming growth factor beta (1) (TGF-β1) were investigated. Also, inflammatory mediators as intracellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1) and tyrosine kinase activity were determined. Low doses of ionizing radiation induced slight activation of the immune cell cytokines (IL-1β and TGF-β1), inflammatory mediators (ICAM, VCAM) and tyrosine kinase activity at doses (0.25, 0.5Gy) reaching to significant levels at 0.75Gy and 1Gy. While, IL-2 and glucose levels were significantly decrease.

Keywords: Gmama-irradiation, IL-1β, IL-2, TGF-β, ICAM and VCAM.

Introduction

Ionizing radiation has a variety of acute and long-lasting adverse effects on the immune system. Whereas measureable effects of radiation on immune cell cytotoxicity and population change have been well studied in human and animal models, little is known about the functional alterations of the surviving immune cells after ionizing radiation. Not only activation of lymphocytes or macrophages, ionizing radiation also can help monocytes/macrophages in finding their targets through activation of their migration and chemotactic behaviour (Lauber et al., 2012).

Glucose uptake and consumption are markedly occurred through activation of T cells mainly during transcriptional regulation process (Wang et al., 2011). Also, Freemerman et al. (2014) stated that activation of lymphocytes is the most reason of glucose spending in immune system.

IL-1β and IL-2 are cytokines involved in early signaling and amplification of inflammatory responses, induction and activation of adhesion molecules, activation of local inflammatory response and regulatory functions of cytotoxic cells. Studies of low doses of radiation exposure, as in atomic radiation workers who occupationally exposed to 0.25 to 10mGy displayed stimulation of both cytokines receptors expression on the surface of peripheral blood lymphocytes taken from normal human donors (Guimbaud et al., 1998 and Xu et al., 1996).

In contrast, the secretion of the anti-inflammatory cytokine TGF-β was enhanced significantly after exposure of radiation at low doses (Rodel et al., 2012). This results in a down-regulation of leukocyte/peripheral blood mononuclear cells (PBMC) adhesion and may contribute to the anti-inflammatory effect of low dose radiotherapy (Roedel et al., 2002).

Tyrosine kinases play a central role in signal transduction, acting as relay points for a complex network of interdependent signalling molecules that ultimately affect gene transcription within the nucleus (Prenzel et al., 2001). Strict regulation of tyrosine kinases activity controls the most fundamental processes of cells, such as the cell cycle, proliferation, differentiation, motility and cell death or survival (Slichenmyer & Fry, 2001). On the other hand, tyrosine kinase activity plays

a critical role in the modulation of growth factor signalling. Activated forms of these enzymes can cause increase in tumor cell proliferation and growth, induce antiapoptotic effects and promote angiogenesis and metastasis (Arora & Scholar, 2005).

Mathias et al. (2015) studied the effects of irradiation on inflammatory/thrombotic plasma markers as ICAM-1 and VCAM-1. The study indicated that low irradiation doses (from 0.025Gy) induce local and systemic changes in an inflammatory-prone genotype. Thereby, high dose rates exerted more pronounced effects than low dose rates, although the general pattern of response was similar. Screening of 19 inflammation-related candidates revealed 11 markers including ICAM-1 and VCAM-1 to be of high value to identify even small late effects of low-dose irradiation.

This study aims to discuss the ability to use low dose of ionizing radiation as a stimulatory factor to enhance the immune system activities and studying its effect on cytokines and tyrosine kinase production.

Materials and Methods

Blood sampling

Blood samples obtained from six healthy volunteers matched groups for ethnic background, sex, smoking and age were also investigated as control group.

All subjects were gave an informed consent for participation in this study. The donors were selected according to current International Programme on Chemical Safety (IPCS) guidelines.

Venous blood were collected under sterile conditions in heparinized vacationer tube (v= 5 ml, Becton Dickinson, USA) containing Lithium heparin as anticoagulant.

Irradiation source:

^{137}Cs γ-rays source was provided by the NCRRT, Egypt, manufactured by the Atomic Energy of Canada. The dose rate was 0.42Gy/min. The samples were kept for one hour at 37°C after irradiation till starting blood separation.

Experimental design

The blood from each volunteer was divided into five groups. One was non-exposed (control group) and the others were applied directly to γ-rays ionizing irradiation with doses (0.25, 0.50, 0.75 and 1Gy). After ionizing irradiation exposure, the blood sample groups incubated at 37°C for 1h before starting biochemical investigations.

Determination of glucose concentration

Glucose concentration measured according to the method described by Anastasovska et al. (2005)

Determination of IL-1β, IL-2

Human IL-1 β and IL-2 were measured according to the method described by Orgenium laboratories (IL- 1β ELISA kit) from AviBion Human IL-1β ELISA.

Determination of TGF-β1

TGF-β1 was determined based on the sandwich principle using a TGF-β1 enzyme-linked immunosorbant assay kit (DRG International, Inc.), according to the manufacturer's instructions.

Determination of inflammatory adherent molecules ICAM-1 &VCAM-1

Human ICAM-1 and VCAM-1in plasma were determined by using ELISA kits from My Bio Source.

Determination of tyrosine kinase activity

Human tyrosine kinase activity was determined by using ELISA kit purchased from SIGMA, ALDRICH for plasma.

Statistical analysis

The obtained data obtained are represented as mean±standard error. Statistical analysis was carried out using (Statistical Package for Social Science) (SPSS) software, significant differences among groups were evaluated using one-way analysis of variance (one-way ANOVA); least-significant difference (LSD) was used for multi-group comparisons. P values ≤ 0.05 were considered as significant

Results

Table 1 showed significant reduction in glucose concentrations of all groups after 1h in 37°C from irradiation. The most significant value noticed in 1Gy group.

Table 2 recorded significant increase in IL-1β

and TGF-β1 levels for all groups, while significant reduction in IL-2 was scored.

Table 3 showed significant increase in both ICAM -1 and VCAM-1 concentration accompanied by increase in tyrosine kinase activity. The most increment value recorded at both 0.75 and 1Gy doses. While, tyrosine kinase level recorded significant increases in its activity at all used doses (0.25, 0.5, 0.75 and 1Gy)

TABLE 1. Glucose concentration in plasma of human blood samples exposed to different doses of ionizing radiation (0.25, 0.5, 0.75 and 1Gy).

	Groups				
	Control group X±SE	0.25Gy group X±SE	0.50Gy group X±SE	0.75Gy group X±SE	1Gy group X±SE
Glucose concentration (μg/L)	110.5±0.3	70.9±0.3[a]	65±0.4[ab]	40.5±0.23[abc]	36.8±1.2[abc]

[a] significant with control.
[b] significant with 0.25Gy
[c] significant with 0.50Gy
[d] significant with 0.75Gy
Statistical significance value (P≤0.05).

TABLE 2. IL-1β, IL-2 and TGF-β1 concentrations in plasma of human blood samples exposed to different doses of ionizing radiation (0.25, 0.5, 0.75 and 1Gy).

	Pro-inflammatory cytokines	Regulatory cytokines	TGF-β1 (pg/ml) X±SE
	IL-1β (pg/ml) X±SE	IL-2 (pg/ml) X±SE	
Control group	41.02±0.07	63.92±0.12	51.3±0.34
0.25Gy group	42.91±0.9[a]	60.33±0.59[a]	55.67±1.19[a]
0.5Gy group	47.32±0.63[ab]	57.02±0.9[ab]	62.13±0.91[ab]
0.75Gy group	78.46±0.18[abc]	41.92±0.14[abc]	89.27±0.67[abc]
1Gy group	80.0±0.32[abcd]	48.02±0.91[abcd]	104.74±1.45[abcd]

[a] significant with control.
[b] significant with 0.25Gy
[c] significant with 0.50Gy
[d] significant with 0.75Gy
Statistical significance value (P≤0.05).

TABLE 3. ICAM-1 and IVCAM-1 levels and tyrosine kinase activity in plasma of human blood samples exposed to different doses of ionizing radiation (0.25, 0.5, 0.75 and 1Gy).

	Adhesion molecules		Tyrosine kinase activity (pg/ml) X±SE
	ICAM-1 (pg/ml) X±SE	VCAM-1 (ng/ml) X±SE	
Control group	54.39±1.42	65±1.15	64.33±0.86
0.25Gy group	53.5±1.0	63.33±0.95	68.61±0.83[a]
0.5Gy group	51.38±0.89	69.83±1.25	71.76±0.98[ab]
0.75Gy group	83.7±0.42[abc]	97.68±3.05[abc]	87.04±0.15[abc]
1Gy group	98.6±1.2[abcd]	109.83±3.9[abcd]	95.44±1.08[abcd]

[a] significant with control.
[b] significant with 0.25Gy
[c] significant with 0.50Gy
[d] significant with 0.75Gy
Statistical significance value (P≤0.05).

Discussion:

It is well known that high doses of ionizing radiation can cause cancer and current regulation of radiation risk is based on the linear no-threshold model which states that even the tiniest dose of radiation will increase your cancer risk. However, it is now an undisputed fact that low doses of radiation (<100mGy) cause different biological responses to that of high doses. Considerable data have also accumulated demonstrating that low doses of ionizing radiation can stimulate mechanisms that can prevent or delay cancer formation. The latter data have been published over many years for many biological endpoints and provide insights into potential new approaches for cancer prevention and treatment, and yet are largely ignored. The current model of radiation risk assessment and the depiction of radiation exposure in the media contribute to radiation phobia in the general public and also in health professionals. Data will be presented to place low doses of radiation into perspective and to highlight the potential of harnessing low dose biological responses for health benefits (Sykes, 2012).

The present study focused on the effects of radiation exposure at doses less than 1Gy to stimulate mechanisms that can defend biological systems against cancer and other immunological defects through the chosen parameters.

Many types of immunological and blood cells are preferentially metabolize glucose as an energy substrate and exhibit a Warburg-like shift (high rate of glycolysis) in cellular metabolism upon activation (Krawczyk et al., 2010; Maciver et al., 2008 and Vats et al., 2006). And this interpreted the obtained results showed in Table 1 which reflected reduction in glucose concentrations of all groups after one hour in 37°C from irradiation. Freemerman et al. (2014) stated that the reduction of glucose concentration in group which exposed to 0.75Gy may be attributed to consumption of glucose due to activation of lymphocytes.

Several studies showed that low irradiation doses (from 0.025Gy) induced local and systemic changes in an inflammatory-prone genotype. Thereby, high dose rates exerted more pronounced effects than low dose rates, although the general pattern of response was similar. Screening of several inflammation-related candidates revealed 11 effective markers (MCP-1, TNF, TGF, ICAM-1, VCAM-1, and fibrinogen, Thy1, collagen IV, CD31) to be of high value to identify even small late effects of low-dose irradiation (Mathias et al., 2015). In that context, the current study investigates some of these markers as (IL-1β, IL-2, TGF-β1, ICAM-1, VCAM-1and tyrosine kinase) with different low doses of irradiation (0.25, 0.50, 0.75 and 1Gy).

Macrophages and mast cells are tissue-resident cells and are frequently the first dedicated immune cells to detect the presence of a pathogen. Both of these cell types have an important role in sensing infection and in amplifying immune responses, through the production of cytokines (interleukins), chemokines and other soluble mediators (such as vasoactive amines and lipids) that have effects on local endothelium and facilitate the migration of other immune cells (such as neutrophils) to the site of an infection through recruitment of latter from the blood (Delves et al., 2017).

In addition, Gasteiger & Kastenmuller (2015) confirmed that radiation exposure stimulate IL-2 receptors expression in a large population of peripheral blood lymphocytes and this shows a triggered-type response rather than being proportional to dose. Also, they demonstrate that normal human cells can respond to doses of radiation in the range of common occupational or medical exposures.

Moreover, low-dose irradiation between 0.3 and 0.7Gy induced a relative maximum of TGF-β production by stimulated endothelial cells and results in a down-regulation of leukocyte adhesion and may contribute to the anti-inflammatory effect of low dose radiotherapy (Roedel et al., 2002).

Our results recorded significant increase in IL-1β and TGF-β1 levels in all groups and these finding could be attributed according to the pervious facts, although significant reduction in IL-2 was scored and interpreted by Chen et al. (2011) and Pandiyan et al. (2011) who discussed the restricting IL-2 during acute infection might additionally enhance immunity and regeneration.

Low radiation doses (0.025 – 0.5Gy) induce adaptive late responses through the enhancement of adherent molecules as ICAM-1 and VCAM-1 expressions which indicate a pro-inflammatory response (Mathias et al., 2015). These conclusions coincide with our results pointed in Table 3.

On the other hand, tyrosine kinase activity plays a critical role in the modulation of growth factor signaling. Activated forms of these enzymes can cause increase in tumor cell proliferation and growth, induce antiapoptotic effects and promote angiogenesis and metastasis. In addition to activation by growth factors, protein kinase activation by somatic mutation is a common mechanism of tumor genesis. Because all of these effects are initiated by receptor tyrosine kinase activation, they are key targets for inhibitors (Arora & Scholar, 2005).

Table 3 demonstrated that tyrosine kinase level recorded significant increases in its activity at all used doses (0.25, 0.5, 0.75 and 1Gy) but these increment levels activate the immunological cells and its processes without proliferation interruption as occurred by high doses exposure. Slichenmyer & Fry (2001) and Vala et al. (2010) confirmed these findings through their work on doses ≤ 0.8Gy which enhance cell migration without impinging on cell proliferation or survival. In addition, low-dose of ionizing radiation induces a rapid phosphorlylation of several cell proteins, including growth factor receptors.

Moreover, tyrosine kinases play a central role in signal transduction, acting as relay points for a complex network of interdependent signaling molecules that ultimately affect gene transcription within the nucleus (Prenzel et al., 2001).

Conclusion

Ionizing radiation has different biological effects according to dose and dose rate. In particular, the biological effect of low-dose radiation is unclear. Low-dose of gamma irradiation activates immune responses in several ways. Low dose ionizing radiation can imitate the role of deteriorate antigen or attenuated microbes as it initiate first defense line of immune response, the step which when lost cancer cells can find suitable environmental condition for growth propagation.

Conflict of interest

The authors declare that: There is no conflict of interest.

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الدور المنشط للجرعات المنخفضة من الأشعاعات المؤينة على استجابة الخلايا الليمفاوية البشرية

شيرين عبد الوهاب مختار منتصر، جيهان رشدى عبد الحميد و أحمد رشاد أبو غدير

قسم بحوث البيولوجيا الأشعاعية ـ المركز القومى لبحوث وتكنولوجيا الأشعاع ـ هيئة الطاقة الذرية ـ القاهرة ـ مصر.

تم تعرض خلايا الدم البشرية إلى جرعات منخفضة مختلفة من الأشعاع المؤين وهى (0.25 و0.50و0.75) جراى ثم تم وضعهم لمدة ساعة فى درجة حرارة الغرفة. ثم تم قياس نسبة السكر لكل من العينات. و قد سجلت النتائج جميعا إنخفاضا ملحوظا فى جميع العينات ولكن الإنخفاض الأكثر سجل عند التعرض للجرعة (0.75) جراى.

وقد أتخذ نقص مستوى السكر فى الدم كعلامة مميزة لنشاط الخلايا الليمفاوية واستهلاكها لنسبة السكر الموجودة بالدم لافراز العديد من الأنترلوكينات و وسيطات الخلايا التى تسهل التواصل بين الخلايا المختلفة.

ومن ثم بدأت باقى القياسات هادفة لتعيين كل من انترلوكين-1و انترلوكين-2 كذلك معامل النمو الورمى. وكل من معاملات الألتصاق لجدار الخلايا VCAM-1 ICAM-1 ونشاط انزيم التيروزين كينيز و استخدامها كدلالات على الدور المفيد والإيجابى للجرعات المنخفضة من الأشعاع المؤين فى تحفيز خلايا المناعة التائية.

وقد سجلت النتائج ارتفاعا ملحوظا فى كل القياسات مدللة على النشاط الذى تعرضت له الخلايا المناعية والدور المحفز للأشعة المؤينة فى الجرعات المنخفضة والجرعة المختارة (0.75) جراى.