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Radioadapted Response Early Effects and Late Effects on Survival of White Blood Cells in Mice (*Mus musculus* L) Post C0-60 Gamma Radiation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Determine the radioadapted response to the early and late effects of white blood cells in mice after Co-60 gamma radiation. Furthermore, the effect of the radioadapted early and late effects of the radioresponse with the interval dose adaptation (DA) with the challenge dose (DC) to the white blood cells of mice, as well as the survival rate of mice white blood cells after gamma Co-60 radiation through the radio-adapted response of the early effect and the late effect.
Place and Duration of Study: The Radioteraphy Installation in Prof. I.G.N.G. NGOERAH Hospital, between from August to October 2022.
Methodology: This research used a low-dose method (adapted-DA dose) and at certain time intervals was continued with a larger dose (dose challenge-DC) in mice with six treatments and one as a control. From each treatment, mice's white blood cells were taken to determine the number of

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leukocytes and their components. Through the number of leukocytes, it is possible to analyze the radio-adaptated response to the early effect and the late effect, so that thesurvival rate of whiteblood cells inmice after gamma Co-60 radiation can be determined.
Results: The results of research on the survival of leukocyte cells, neutrophils, and lymphocyte cell components show early-responding tissue and late-responding tissue. As for cell survival, other componentssuch asmonocytes, eosinophils, andbasophils did not respond in the same way after gamma Co-60 radiation. In general, giving a time delay to the challenge dose for all radiation dose treatments gives a fast response (early effect) to cell survival within a few days after radiation, then with time the full response that occurs is a slow (late effect). The longer the delay in giving the challenge dose, the slower the response will fully occur in the tissue after gamma Co-60 radiation. The cell survival curve also shows that the α/β ratio for the early effect is 3 and for the late effect is 10, which is an illustration of the early effect on radiation, with a linear shape of the cell death curve indicating that tissue is more sensitive to changes in radiation dose and has a greater α/β ratio. When compared to the tissue with a late effect on radiation, the tissue has a smaller α/β ratio indicating that the tissue is less sensitive to changes in the timing of the challenge dose.
Conclusion: Radioadapted response to the survival of leukocytes, neutrophils cell components, and lymphocytes have shown early responding tissue and late responding tissue after gamma Co-60 radiation. By giving a time lag for the challenge dose, an early response to cell survival occurs for only a few days, then with time, fully the response that occurs is late responding.

Keywords: Adaptation dose; challenge dose; early effect; late effect; cell survival.

1. INTRODUCTION

Low-dose radiation induction can cause changes in the mechanisms of cellular and molecular systems. Under certain conditions, cells can be protected against the effects caused by subsequent high-dose radiation [1]. This term is called the adaptation response, which is a response that causes changes in gene expression. These changes occur at low-dose radiation exposure of approximately <0.5 Gy. Changes in gene expression under certain circumstances can protect cells against the effects that arise from subsequent radiation exposure with higher doses [2,3].

The results of the radiation-induced micronuclei test showed that cells can repair broken DNA breaks. Research on human skin cells has been carried out to determine exposure to low doses and dose rates on the ability of cells to repair chromosomal breaks. The frequency of micronuclei per cell in an adapted radiation dose was 0.5 Gy at a dose rate of 2.5 mGy/minute. Radiation was carried out again at a dose of 4 Gy at a dose rate of 1.8 Gy/minute after 0 hours and 5 hours. The results showed that low doses and dose rates can stimulate cells to improve repair against chromosomal damage, resulting in a reduction in subsequent radiation exposure.

Important biological variables can be shown that chromosomal damage is not always proportional to the dose [4]. Research by Widyasari in 2013, with an adapted dose of 0.1 Gy followed by doses of 1 Gy, 2 Gy, and 3 Gy was able to stimulate cells to increase their ability to repair cells (recovery) after Co-60 radiation. From day 1 to day 30 after Co-60 radiation, the number of leukocytes decreased, while from day 30 to day 60, the number of leukocytes increased [5]. The results of this study were different from studies with doses of 1 Gy, 2 Gy, and 3 Gy without dose adaptation, where the rate of decrease in the number of leukocytes from day 1 to day 30 was faster than using dose adaptation [6]. It can be stated that, without an adaptation dose, radiation has a damaging effect on stem cells and precursor cells in the bone marrow thereby reducing the number of blood cells in its distribution [7]. According to Hall (2003) and Mayani (2013), the radioadaptation response is a biological phenomenon that shows resistance to radiation by administering one or several initial radiation doses at very low levels [8]. The adaptation response in the radiation field is synonymous with radioresistance which is able to change the biological effectiveness of the next radiation with a larger dose. Cell protection against radiation exposure is determined by the survival rate of these cells. One of the characteristics that determine the level of cell survival is the radioadapted response of the early effect and the late effect of the cell [9]. Thus it is necessary to study the radioadaptation response to the early and late effects of white blood cells in mice after gamma Co-60 radiation.

2. METHODS

2.1 Place and Duration of Study

The study was conducted from August to October 2022 at the Radioteraphy Installation of RSUP Prof. I G N G Ngoerah Hospital.

2.2 Research Procedure

2.2.1 Radiation process

The radiation process wascarried out by giving an adaptation dose (DA) of 0.1 Gy to alltreatment groups except thecontrol group. Then given achallenge dose (DC) of 3 Gy at intervals (t) 0, 1, 2, 3, 4, and 5 hours. The following are the steps of the radiation process carried out:

a. Mice were placed in cages marked as control and with DA-DC intervals at 0 hours, DA-DC intervals at 1 hour, DA-DC intervals at 2 hours, DA-DC intervals at 3 hours, DA-DC intervals at 4 hours, and DA-DC intervals at 5 hours with each cage containing 5 mice.
b. The cage containing mice according to the treatment was put into the radiation room and placed on the treatment table.
c. The distance between the radiation source and the mouse object was set at 80 cm under constant SSD conditions.
d. The operator regulates the radiation process through the control panel and observes the radiation process through a television screen connected to the camera in the radiation room.
e. After the first radiation process was completed, the mice cages were removed from the radiation room and then continued with the second cage until the fifth cage with the same steps.

2.2.2 Retrieval and counting of white blood cells [10]

The leukocyte sampling process is carried out with the following steps:

1. Blood sampling for each mouse is carried out by giving an anesthetic solution first to the eye.
2. Then after 5 minutes, start the process of taking blood by holding the nape of the neck to reduce the movement of the mice.
3. Gently insert the capillary pipette into the vein in the eye.
4. Blood will come out through a capillary pipette which is then placed in the EDTA tube until ±0.5 cc of blood is obtained. Then the blood in the tube is shaken so that the blood and EDTA are evenly mixed.

The blood sample is sucked from the EDTA tube using a hemocytometer pipette. Then the Turk solution is also sucked using a hemocytometer pipette.

1. This is a dilution process with a ratio of blood sample and Turk solution is 1:50. The goal is to destroy red blood cells so that only leukocytes are visible in the microscope.
2. Then themixture of blood and Turk solution is placed in a shaker for 5 minutes.
3. The glass object is given distilled water first so that it can stick to the hemocytometer. Then the mixed blood is put into thehemocytometer.
4. Then do the calculation of the number of leukocytes using a microscope and laboratory counter.

2.2.3 Data processing and data analysis

a. Data processing

Data processing starts from the editing, coding, entry, and tabulating stages with the SPSS (Statistical Product and Service Solutions) program for Windows version 17.

b. Data analysis

After the data is tabulated, descriptive analysis is then carried out. Before the analysis test is carried out, a normality test is first carried out for normally distributed data. To analysis the differences used analysis ofvariance (ANOVA). ANOVA isbetter known asthe F-test (Fisher's Test) at $\alpha = 5\%$, tosee thesignificant difference between eachdose ofadaptation tothe dose of challenge of white blood cells (leukocytes).

3. RESULTS AND DISCUSSION

The resultsof calculating the number of leukocytes for control and treatment mice can be shown in Tables 1 and 2. In this study, mice irradiated at a challenge dose of 3 Gy had a lower dose range than 7 Gy, which is the LD50/30 value of mice, as shown in Table 1.

Table 1. Leukocyte cell survival results for treatment DA (0.1 Gy) with DC(3 Gy)

Treatment	Survival Leukocytes Cell (/mm³)			
	1	10	20	30
Control	7.825	7.925	7.975	8.175
P1 (0 hours)	4.460	3.890	2.798	1.841
P2 (1hours)	5.330	4.920	3.670	2.620
P3 (2 hours)	6.110	5.826	4.670	3.570
P4 (3 hours)	6.851	6.481	5.424	4.459
P5 (4 hours)	7.331	6.605	5.849	5.002
P6 (5 hours)	7.505	6.974	6.320	5.612

Table 2. Survival results of leukocyte component cells for treatment interval DA (0.1 Gy) with DC(3 Gy)

a. Neutrophil

Treatment	Survival Neutrophil Cell (%)			
	1	10	20	30
Control	74,02	73	73,75	73,85
P1 (0 hours)	51,01	48,4	38,78	28,67
P2 (1hours)	56,56	52,89	44,65	37,4
P3 (2 hours)	62,12	58	50,6	43,23
P4 (3 hours)	66,56	61,45	55,98	50,68
P5 (4 hours)	69,85	65,4	61,6	58,04
P6 (5 hours)	73 69	73	66,76	63,67

b. Lymphocytes

Treatment	Survival Lympocytes Cell (%)			
	1	10	20	30
Control	22,35	22,35	21,49	21,69
P1 (0 hours)	14,97	14,02	11,43	8,72
P2 (1hours)	16,75	16,04	13,67	11,07
P3 (2 hours)	18,19	17,25	15,02	13,03
P4 (3 hours)	19,87	18,97	16,98	15,49
P5 (4 hours)	21	19,67	18,09	16,89
P6 (5 hours)	22,22	21,09	19,42	18,06

For leukocyte components suchas monocytes, eosinophils andbasophils do not show behavior in accordance with leukocytes. Then an analysis was carried out to determine the early and late effects on the survival of leukocyte cells and leukocyte components suchas neotrophils, lymphocytes, monocytes, eosinophilsand basophils. The early effect and the late effect can be determined by knowing the α/β ratio which can be shown by graphing the α/β ratio of the early effect and the late survival effect of leukocyte cells and leukocyte components respectively in Figs. 1-3.

The early and late effects on normal tissue are highly dependent on the renewal of each tissue, which is associated with cell differentiation, death and regeneration. Where there are differences in the kinetics of each cell type in the tissue to the pathogenesis of the response [11]. This difference gave rise to the terms early responding tissue and late responding tissue. Early responding tissue is a tissue that has a fast reaction to radiation, while late responding tissue is a tissue that has a slow reaction to radiation. In a tissue or even an organ composed of various kinds of cell components, which allows for the occurrence of more than one response in the same tissue [12].

The results of the research as shown in Figs. 1-3 show the survival of leukocyte cells, neutrophil cell components, and lymphocytes which show an early responding tissue and a late responding tissue response. Whereas for cell survival, other

components suchas monocytes, eosinophils, and basophils did not respond in the same way after post mortem Co-60 gamma radiation. In general, giving a time delay to the challenge dose for all radiation dose treatments gives a fast response (early effect) to cell survival within a few days after radiation, then with time the full response that occurs is slow response (late effect). The longer the delay in giving the challenge dose, the slower the response fully occurs in the tissue after gamma Co-60 radiation [13]. According to research conducted by Lusiyanti, and Syaifudin (2008), the results of this study also show the same thing where the early effect occurs within 1-2 weeks after radiation and occurs in cells that proliferate rapidly, but with the delay in giving the challenge dose the longer the dominant late effect occurs or it can be said that late responding tissue is more sensitive to changes in the time of administration of the challenge dose [9].

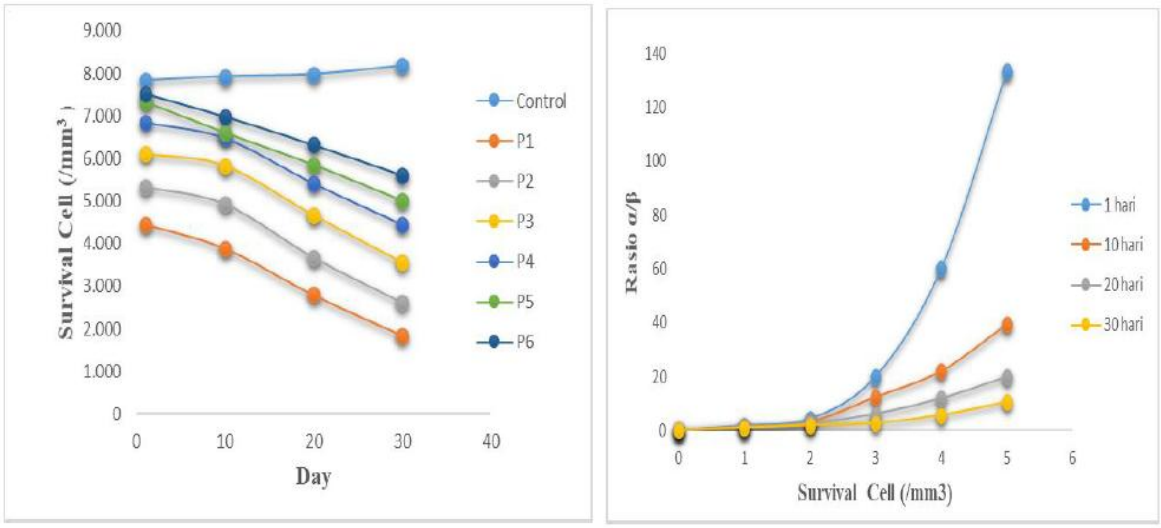

Fig. 1. Survival of leukocyte cells after gamma Co-60 radiation and α/β ratio of early and late effects

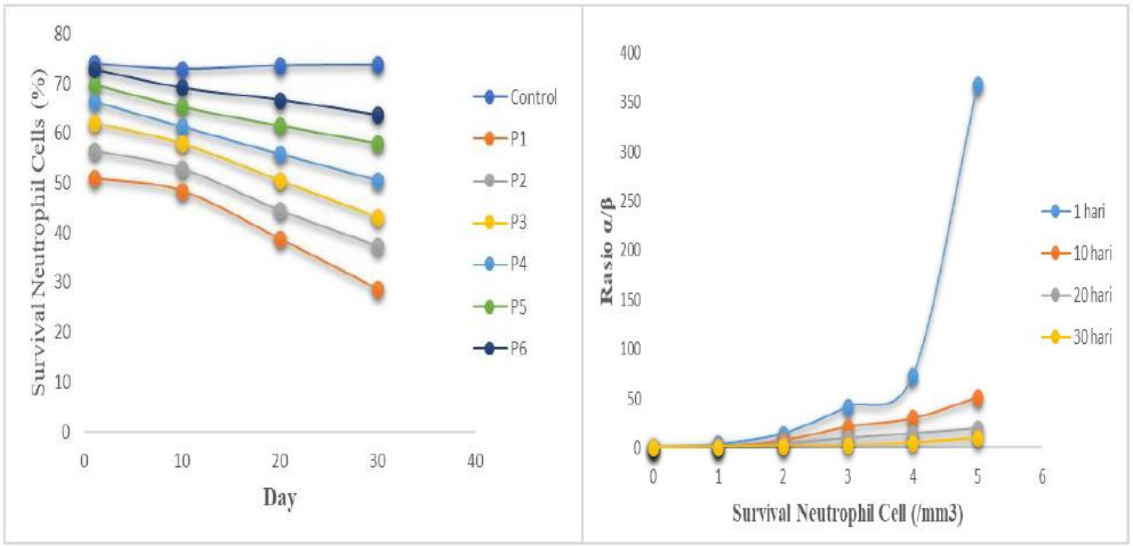

Fig. 2. Neotrophic cell survival after gamma Co-60 radiation and α/β ratio of early and late effects

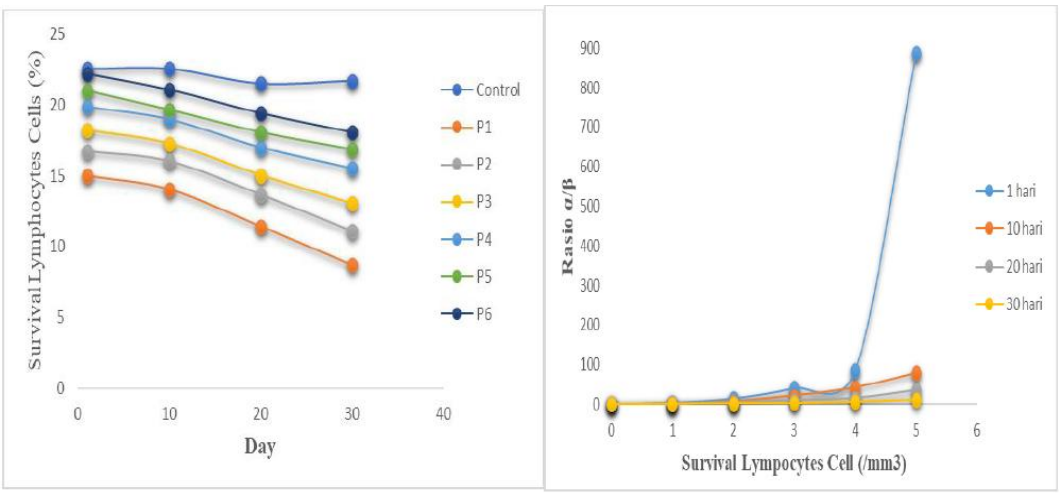

Fig. 3. Lymphocyte cell survival after gamma Co-60 radiation and α/β ratio of early and late effects

In Figs. 1-3, a quadratic linear model cell survival curve (LQ curve) is formed which shows the value of the α/β ratio, where the linear curve is depicted with a straight line/arithmetic, which shows that cell death is directly proportional to the dose (α). Another component illustrates that cell death is directly proportional to the square of the dose, which is called the quadratic component (β). The linear section illustrates that low doses can cause double-strand breaks to occur due to the interaction of single electrons in the absorption of Co-60 gamma rays. The likelihood of these rapid chromosomal/DNA aberrations occurring is directly proportional to the dose. So the greater the dose given, the more damage will occur [7]. At higher doses, the termination of two DNA chains can occur due to two different electrons. The likelihood of this interaction is proportional to the square of the dose administered. The results also show that the α/β ratio for the early effect is 3 and for the late effect is 10. In the figure, the tissue with an immediate/early response to radiation, with a linear cell death curve shape shows that the tissue is more sensitive to changes in radiation dose and has a larger α/β ratio. When compared to tissues that respond indirectly/late to radiation, these tissues have a smaller α/β ratio indicating that the tissue is less sensitive to changes in the timing of the challenge dose [14-16].

4. CONCLUSION

The radioadapted response to the survival of leukocyte cells, neutrophil cell components, and lymphocytes have shown an early-responding tissue response and a late-responding tissue response. As for cell survival, other components such as monocytes, eosinophils, and basophils did not show the same response after Co-60 gamma radiation. By giving a time lag to the challenge dose for all radiation dose treatments it gives an early response to cell survival within a few days after radiation, then with full time the response that occurs is a late response, and the value of the α/β ratio for the early effect is 3 and the late effect is 10 which is a value that is still representative for mammals.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cunningham JR. The physics of radiology. Charles C Thomas Publisher. USA; 1983.
2. Lusiyanti Y, Alatas Z. dan Indrawati I. Detection of dicentric and translocation

chromosomes in lymphocytes of radiation workers. Center for Radiation Safety Technology and Metrology. BATAN; 2013.
3. Nengsi P, Abdullah B, Satrial M. Measurement of Radiation Exposure to Brachytherapy Unit Radiation Workers Using a Thermoluminescent Dosimeter (TLD). Undergraduate Thesis, Physics, FMIPA Hasanuddin University; 2016.
4. Mitchel REJ, Boreham DR. Radiation protection in the world of modern radiobiology: Time for A new approach. Proceedings of 10th International. Congress of the International Radiation Protection Association; 2000.
5. Widyasari L, Sutapa GN, Sudarsana WB. Radioadapted Response to the Quantity of Leukocyte Components of Mice (*Mus Muscullus* L) with Gamma Co-60 Radiation. Physics Buletin, Udayana University; 2013.
6. Asari AD, Sutapa GN, Sudarsana WB. Determination of leukocyte quantity of mice (*Mus muscullus* L) at the lowest point post-gamma Co-60 radiation. Physics Buletin. Udayana University; 2013.
7. Cember H. Introduction to health physics. First Edition. Pergamon Press Northwestern University. Oxfrord New York; 2014.
8. Hall EJ. Radiobiology for the Radiologist. 5th ed. Lippincott Wlliams & Wilkins, Philadelphia. 2000;30-45.
9. Hall, EJ. The Bystander Effect. Health Physics. 2003;85:31-35.
10. Mayerni Ahmad A, Abidin Z. The impact of radiation on the health of radiation workers at arifin ahmad hospital, santa maria hospital and Awal Bros Hospital Pekanbaru. Journal of Environmental Science. Riau Provincial Health Office, Jl. Cut Nyak Dien III Pekanbaru. 2013;7(1).
11. Lusiyanti Y, Syaifudin M. Application of the interaction effects of radiation with biological systems as a biological dosimeter. Center for Radiation Safety Technology and Metrology. BATAN; 2008.
12. Depkes RI. Health center laboratory examination instructions. Health Laboratory Center of the Directorate General of Community Health; 2015.
13. Wouters BG. Cell death after irradiation: how, when and why cells die. In: Michael J, Albert vdK, editors. Basic Clinical Radiobiology. 4th ed. London: Hodder Arnold, Chapter. 2009;3:27-39.
14. Effendi Z. the role of leukocytes as allergic anti-inflammatory in the body. Department of Histology, Faculty of Medicine, Sumatra Utara University; 2003.
15. Milan TJM, Stell GG. Molecular aspects of radiobiology In: Steel GG, editor. Basic Clinical Radiobiology. London: Edward Arnold. 1993;211-214.
16. Scarpato R, Lori A, Tomei A, Cipollini M, Baralen R. High prevalence of chromosome 10 rearrengements in human lymphocytes after *In vitro* X-ray irradiation. International Journal of Radiation Biology. 2011;76(5):661-666.

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