



Survival of White Blood Cells of Mice (*Mus Musculus L*) Against Adaptation Dose of Gamma Co-60 Radiation



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Abstract

Several studies on the effects of low dose radiation have shown a shift in the three main paradigms of radiation biology. One of them is a phenomenon known as adaptation response. The phenomenon of adaptation response is a response that occurs when changes in gene expression can be induced by exposure to low doses of radiation around <0.5Gy. Changes in gene expression in certain circumstances serve to protect cells against the effects of subsequent radiation exposure with higher doses (dose challenges). The purpose of this study was to obtain a radiotherapy method that could show a reduction in patient dosage. In this study, the sample of mice (*mus musculus l*) was divided into 6 groups, namely 1 control group and 5 treatment groups. Five treatment groups were given an adaptation dose of 0.05Gy for treatment I; 0.10Gy treatment II, 0.15Gy treatment III, 0.2 Gy treatment IV, and treatment V; 0.25Gy. Furthermore, all treatments were immediately given a challenge dose 3Gy. The results of the analysis showed that the lowest leukocyte cell survival occurred on day 30 and returned to normal conditions on day 60 after gamma Co-60 radiation.

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1 Introduction

Several studies on the effects of low dose radiation have shown a shift in the three main paradigms of radiation biology. One of them is a phenomenon known as adaptation response (Alatas, 2006). The adaptation response is the induction of a low radiation dose that allows changes to the mechanisms of the cellular and molecular systems. Under certain conditions, it can protect cells against the effects caused by the next high radiation dose received (Olivieri et al., 1984). The phenomenon of radio adaptation response is a response that occurs when changes in gene expression can be induced by exposure to low doses of radiation around <0.5 Gy. Changes in gene expression in certain circumstances serve to protect cells against the effects caused by subsequent radiation exposure at higher doses (Brooks et al., 2005; Kadhim et al., 2004).

This research was conducted on four sample groups of mice irradiated with gamma Co-60 with a dose challenges of 1 Gy; 1.5 Gy; 2 Gy; 2.5 Gy and 3 Gy. In the fourth group of mice, the five doses of the challenges were given 5 hours after receiving the adaptation dose of 0.1 Gy. The result was that the lowest decrease in the number of leukocytes that occurred in the fourth sample group of mice. This indicates that the adaptation response increases when there is a time interval between the dose of adaptation and the dose of challenges (Hall, 2003). Also, this study used an adaptation dose of 0.1 Gy, followed by a dose of 1 Gy, 2 Gy, and 3 Gy which was able to stimulate cells to increase their ability to recover cells after Co-60 radiation. The quantity of leukocytes has decreased from day 1 to day 30. Furthermore, after passing the 30th day to the 60th day the quantity of leukocytes has increased (Widyasari, 2019). These results differ from the number of leukocytes in mice with the treatment doses of 1 Gy, 2 Gy, and 3 Gy without the dose adaptation. This shows the rate of decrease in the number of leukocytes from day 1 to day 30 which is faster without an adapted dose compared to using an adapted dose (Chan et al., 2010). It can be stated that, in the absence of an adapted dose, radiation has a damaging effect on stem cells and precursor cells in the bone marrow thereby decreasing the number of blood cells in their spread (Haley, 1965).

According to Okazaki & Gotoh (2005); Kadhim et al. (2004), radio adaptation response is a biological phenomenon where resistance to radiation is obtained by administering one or more initial radiation at a very low dose. In the radiation field, the adaptive response becomes a synonym for radioresistance which can alter the biological effectiveness of subsequent radiation at a larger dose. To find out cell survival, it is necessary to do several adaptation doses or low doses before giving a larger dose (Sun et al., 2014). Based on these results, it is necessary to research the response of several adaptation doses to the number of leukocytes of mice (*Mus musculus L*) with gamma Co-60 radiation to determine cell survival as a basis for determining radiotherapy methods (Evans et al., 1978; Hucklebridge et al., 1981).

2 Materials and Methods

Radiotherapy was carried out at the Sanglah Hospital Radiotherapy Installation using a Co-60 Teletherapy aircraft and blood tests for mice were carried out at the Bali Provincial Laboratory. Radiation process by giving several doses of adaptation starting from 0.05 Gy; 0.1 Gy and 0.15 Gy in all groups of mice except the control group and immediately given a challenge dose of 3 Gy. After the implementation of the radiation is complete, it is followed by taking and counting the number of white blood cells (*leukocytes*) and their components by taking a blood sample of mice through a vein in the eye. The blood is compressed into the EDTA tube until ± 0.5 cc is obtained and 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. The Turk solution is also sucked using a hemocytometer pipette. This is a dilution process with the ratio of a blood sample to Turk solution being 1:50. The goal is to destroy the red blood cells so that only leukocytes can be seen on the microscope. Then the results of the mixture of blood with the Turk solution are put on a shaker for 5 minutes. Furthermore, the mixed-blood is entered into the hemocytometer. The number of leukocytes was observed under a microscope and counted by a laboratory counter.

Before the analysis test is carried out, the normality test is carried out for normally distributed data. ANOVA statistical analysis (analysis of variance) was used to analyze the differences. ANOVA is better known as the F-Test (Fisher Test) at $\alpha = 5\%$, to see the significant difference in each dose of adaptation to the challenge dose of leukocytes and its components.

3 Results and Discussions

The results of the calculation of the leucocyte quantity of mice for control and treatment can be represented in a graphical form as shown in Figures 1, 2, and 3. In this study, mice irradiated at the 3 Gy challenge dose is a dose range lower than 7 Gy which is the LD50 / 30 value of mice.

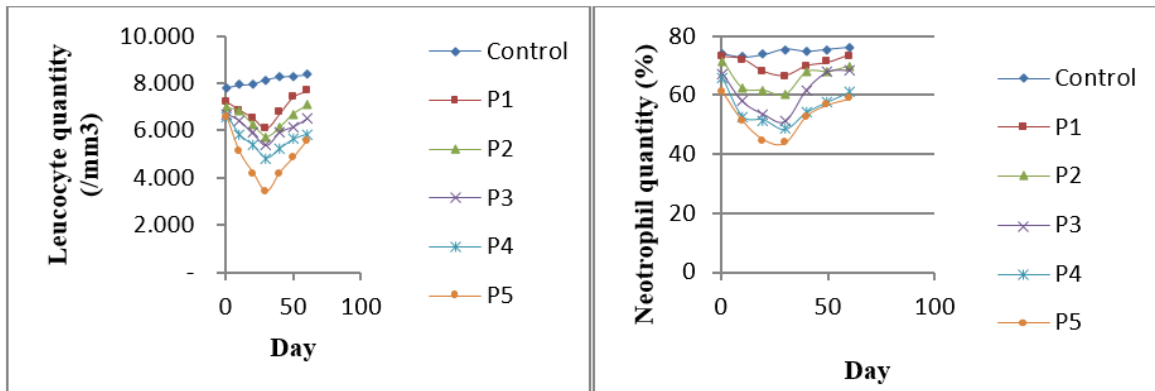


Figure 1. Quantity of leucocytes

Figure 2. Quantity of neutrophil components

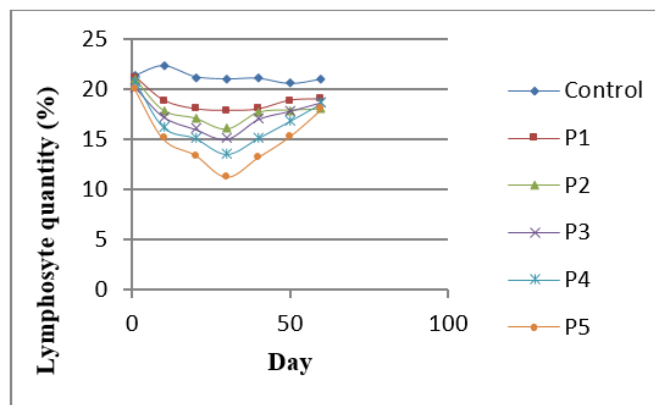


Figure 3. Quantity of lymphocyte components

According to the definition of LD 50/30 (lethal dose) is a dose that causes 50% of the population of mice to die within 30 days. For mice to stay comfortable and healthy during the study, the maximum radiation dose was chosen 3 Gy, with a fixed-dose rate of 0.996 Gy/minute. Thus, radiation will reduce the number of blood cells that depend on radiosensitivity and cell survival. The lowest quantity of control leukocytes in this study was $7,825 \times 10^3/\mu\text{l}$, which is the quantity of leukocytes that is still in the normal limit, which ranges from $4-11 \times 10^3/\mu\text{l}$. The post-radiation leukocyte quantity with the adaptation dose treatment and immediately given the 3 Gy challenge dose, showed that there was a decrease in the leucocyte quantity until the 30 days and showed an increase again after the 30 days until it reached normal again on the 60 days. known to have a damaging effect on stem cells and precursor cells in bone marrow (bone marrow syndrome), thereby reducing the number of blood cells in their circulation. When this sharp decrease in quantity can cause various effects that can be experienced, such as nausea, vomiting, tongue feeling tingling, even the worst it can cause baldness (Alatas, 2006).

With dose adaptation treatment, a biological phenomenon that is resistant to radiation will occur. Resistance is obtained from one or more initial radiation exposures at very low doses. Radio adaptation response can change the effectiveness of subsequent radiation exposure with a larger dose (Khadim et al., 2004). The fact that this study resulted in the achievement of better cell survival after day 30 to day 60 due to the treatment of several adaptation

doses before then giving a challenge dose of 3 Gy. Also, the rapid rate of increase is influenced by the recovery process (recovery) cells due to the effects of radiation that lasts over time.

Figures 2 and 3 are graphs of the results of research on leukocyte components, namely the quantity of neutrophils and lymphocytes, which show an image and response that is almost the same as the quantity of leukocytes. According to [Lusiyanti & Syaifudin \(2008\)](#), these two components of leukocytes can be used as general hematopoietic indicators of radiation exposure. However, this is not the case for other leukocyte components such as monocytes, eosinophils, and basophils as shown in Figures 4,5 and 6 below.

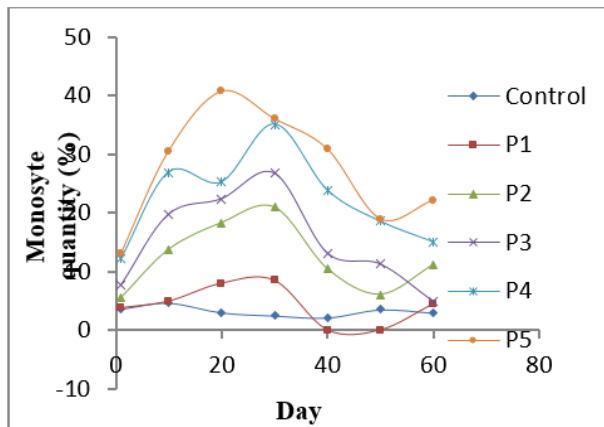


Figure 4. Quantity of monocyte components

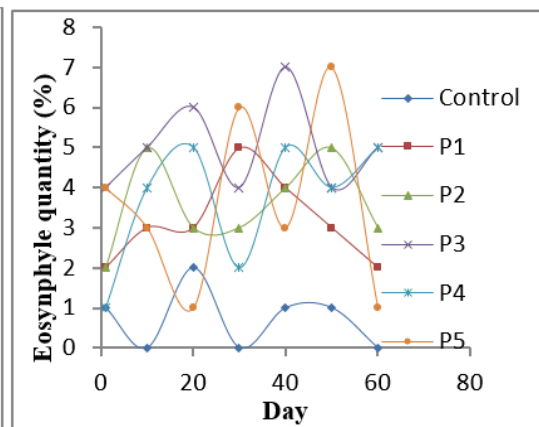


Figure 5. Quantity of eosinophil components

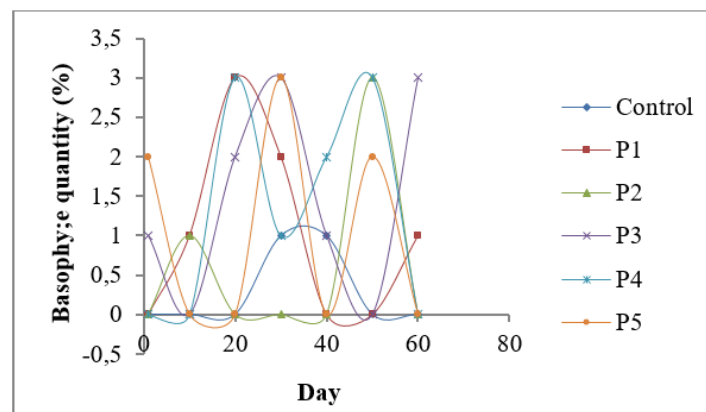


Figure 6. Quantity of basophil components

The three quantity components of this leukocyte give a very different response from the quantity of leukocytes. Where the three quantities of this leukocyte component show a fluctuating response, according to the function of monocytes, eosinophils, and basophils as phagocytes, tend to have almost the same response to radiation induction ([Hall, 2000](#)). The statistical analysis carried out supported the results of this study that the quantity of leukocytes, the quantity of neutrophil and lymphocyte components had a significant difference at a significance of 0.05 with the other three quantities of leukocyte components. According to [Moscalev \(2011\)](#), the two components of leukocytes are the most sensitive compared to the other three components of leukocytes.

4 Conclusion

Leukocyte cell survival, neutrophil, and lymphocyte components provide a clear picture of cell development from day to day after dose adaptation treatment of Co-60 gamma radiation. The picture of cell survival is indispensable in determining radiotherapy methods that emphasize low doses or dose adaptations.

Conflict of interest statement

The authors declared that they have no competing interests.

Statement of authorship

The authors have a responsibility for the conception and design of the study. The authors have approved the final article.

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