Postirradiation white blood cell recovery in rats: Following single and double (repeated) X-ray exposure

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Abstract

Purpose: This study aims at determining the effect of radiation on peripheral white blood cells (WBCs) and the time for their full recovery in Albino Wister Rats following single and double (repeated) X-ray exposure so that a safe period for repeated irradiation can conveniently be recommended for any radiographic examination.

Materials and Methods: Twenty one healthy albino Wister Rats of aged between 2–4 month and weighing about 140–200 g were used for this study. They were divided into three Groups A, B, and C. Group B and C were irradiated with X-ray from a diagnostic X-ray machine. Group C was immediately irradiated again with the same exposure factors at about 3–4 min interval. Blood samples from both control and experimental groups (after irradiation) were collected through a period of 30 min to 7 days and subjected to standard hematologic examination to determine the WBC count and differential leukocytes count.

Results: A sharp fall in WBC was observed in the single-exposed group 30 min following irradiation. This is however more pronounced in the double-exposed group. This fall persisted till 48 h after irradiation. The WBC count returned to normal on the 3rd and 4th day following irradiation for both experimental groups. The maximum repair and recovery recorded were 98.6% for single exposure and 95.9% for double exposure of the normal count.

Conclusion: This study has presented that the recovery of an irradiated peripheral WBC is not 100% and that the effect is more remarkable in cells with double (repeated) irradiation. A safe period of at least 3–7 days should be allowed for the cells to recover from previous irradiation before an examination is repeated. This will serve as a guide to radiographers/radiologist in the management of patients who need follow-up examinations.

Keywords: Irradiation, rats, recovery, white blood cell

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INTRODUCTION

The effects of ionizing radiation on human tissue/cells have long being studied. Few months after the discovery of X-ray by Wilhelm Conrad Rontgen in 1895, the first case of human injury resulting from radiation exposure was reported.[1] The formation of free radicals by ionizing radiation results in harmful biological effects in the body whose rate of impact is determined by the total dose absorbed, the rate at which the dose is delivered, and the quality of the radiation.[2] Depending on these three parameters, radiation exposure in the immediate aftermath could lead to a myriad of deleterious effects, including acute

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radiation syndrome.[3] Hall and Giaccia, 2006 observed that there is a continues decline in blood counts at higher radiation dose, due to the death of some of the bone marrow stem/progenitor cells which can result in lethality. They also showed that blood cell counts decline at lower radiation doses until surviving precursor cells proliferate to restore balance.[4] It was observed by Williams and McBride, 2011 that individuals are at increased risk of infection and hemorrhage during this period of declining blood cell counts.[5]

In general, actively dividing cell are the most sensitive to cell killing by radiation. At lower radiation doses below 1.0 Gy (100rad), the damage done is reasonable, and most of the cells recover or survive, however, there may be subject to subsequent malignant changes.[6] Although, successes have been recorded in medical diagnosis and treatment using X-radiation, its genetic and hematopoietic effects remains controversial.[7]

Radiation-induced hematological alterations have been extensively studied with doses ranging from 2 up to 50 Gy. Lymphocytes are among the most radiosensitive cells in the living organisms. They are involved in immunological responses and are of immense interest to researchers and clinicians because of their extreme sensitivity to ionizing radiation.[8] Therefore, this study was designed to investigate the postirradiation cell recovery of in peripheral white blood population after whole body irradiation in order to determine the time for full recovery and to hypothesize a safe period for repeat exposure in patients requiring series of radiologic examination

MATERIALS AND METHODS

A total of 21 albino rats aged between 2–4 months and weighing between 140–200 g were conveniently separated into three Groups; A, B, and C. Group A serves as the control group, Group B was subjected to single radiation exposure while Group C recieved double (repeated) radiation exposure. Group B and C were irradiated with X-ray dose of about seventy kilovolt (70 kV) on 12.5 mAs, which is within the diagnostic range of 2–3 mGy per fraction, from X-ray machine MS-185, serial no. 0904 GE at focus to film distance of 90 cm. Group C was immediately irradiated again with the same exposure factors at about 3–4 min interval. Blood samples were taken from the control and experimental groups and subjected to standard white blood cell (WBC) count, and differential leukocytes count as described by Barr *et al.*[9] All data were recorded appropriately for further analysis.

Blood sample collection

Blood sample was collected from the tail vein and transferred to a specimen bottle containing ethylenediamine tetraacetic acid anticoagulant. The blood samples were collected per interval of 30 min, 24 h, 48 h, 3, 4, 5, and 7 days postirradiation of the experimental group. Blood samples were also collected during same time interval for the control group.

Data presentation and analysis

All data generated during sampling and laboratory examination of blood samples were recorded appropriately and analyzed for statistical significance using Chi-square test statistics at 95% confidence interval. $P \leq 0.05$ were regarded as significantly different. With the data obtained, postirradiation cell recovery curve was also plotted.

RESULTS

The result in Table 1 shows the WBC count for both the control and experimental groups (single and double exposure). A sharp fall in WBC from 7.10 to 5.50 count/L was observed in the single-exposed group 30 min following irradiation. This is even more pronounced in the double-exposed group with the value 4.90 count/L. This fall persisted till 48 h after irradiation. The WBC count returned to normal on the 3rd and 4th day following irradiation for both experimental groups.

From the result of Table 2, the neutrophils count was also observed to drop in the first 30 min after irradiation from 2.81 count/L to 2.75 count/L for single exposure, and 2.35 count/L for double (repeated) exposure. The fall in neutrophil count persisted till the 3rd and 4th day on which it returned to normal. The drop in lymphocyte count was also remarkable within the first 24 h in both experimental groups, from 4.18 counts/L to 2.53 count/L and 2.35 count/L for single and double (repeated) exposure, respectively. This fall was quickly restored within the first 48 h for single exposure and 3 days for double exposure. Eosinophils and monocytes concentrations also showed sharp decrease in the first 24 h in both experimental groups after which they regained gradually.

Table 1: Total white blood cell count of albino Wister rats following single and double exposure

Groups	WBC count/L (10^9) Periods						
	30 min	24 h	48 h	3 days	4 days	5 day	7 days
Group A (control)	7.10	7.10	7.00	7.30	7.00	7.10	7.95
Group B	5.50[a]	4.60[a]	5.60[a]	7.20[b]	7.10[b]	6.30[a]	7.30[a]
Group C	4.90[a]	4.30[a]	4.45[a]	7.00[b]	6.80[b]	6.00[a]	7.00[a]

[a]Values not significantly different from control ($P>0.05$); [b]Values significantly different from control ($P<0.05$). WBC – White blood cell

Table 2: Percentage distribution of leucocytes indices of control and experimental groups (single and double exposure)

Periods	Differential leukocyte count				
	Groups	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
30 min	A1	2.81	4.18	0.14	Nill
	B1	2.75[b]	2.53[a]	0.11[b]	0.11
	C1	2.35[a]	2.35[a]	0.10[b]	0.10
24 h	A2	2.99	3.72	0.58	Nill
	B2	2.39[a]	1.75[a]	0.28[a]	0.18
	C2	2.58[a]	1.35[a]	0.26[b]	0.09
48 h	A3	3.64	2.66	0.42	0.28
	B3	2.35[a]	2.69[b]	0.45[b]	0.11[a]
	C3	2.73[a]	2.29[a]	0.33[b]	0.11[a]
3 days	A4	4.60	2.48	0.29	Nill
	B4	3.89[a]	2.88[a]	0.29[b]	0.14
	C4	2.24[a]	4.20[a]	0.42[a]	0.14
4 days	A5	4.06	1.96	0.70	0.28
	B5	2.98[a]	3.98[a]	0.14[a]	Nill
	C5	4.08[b]	2.04[b]	0.54[b]	0.14[a]
5 days	A6	4.40	2.13	0.43	0.14
	B6	2.77[a]	3.28[a]	0.25[a]	Nill
	C6	3.64[a]	2.80[a]	0.42[b]	0.01[a]
7 days	A7	3.82	3.34	0.64	0.16
	B7	2.77[a]	4.23[a]	0.29[a]	Nill
	C7	3.92[b]	2.80[a]	0.28[a]	Nill

[a]Values not significantly different from control ($P>0.05$); [b]Values significantly different from control ($P<0.05$)

The recovery was, however, faster in single exposure than in the double (repeated) exposure.

The result of Figure 1 shows the relationship in WBC count between the control and the experimental groups (single and double exposures). There is a remarkable decline in the WBC count in both experimental groups, although more pronounced in the double-exposed groups than in the single-exposed groups. However, maximum repair and recovery were observed in both experimental groups between 3rd and 4th days after the exposure.

The curve of Figure 2 shows a remarkable destruction of WBC generally and lymphocytes specifically and a gradual recovery process for the single-exposed groups. The destruction of neutrophils was gradual and a faster recovery process was observed. In general, the three curves (for WBC, neutrophils, and lymphocytes) show maximum repair and recovery between 3rd and 4th days after exposure to radiation.

This curve Figure 3 also shows the rapid destruction of WBC generally and lymphocytes specifically and a gradual recovery process for the double-exposed groups. The destruction of neutrophils was gradual and a faster recovery process was observed. In general, the three curves (for WBC, neutrophils, and lymphocytes) show maximum repair and recovery between 3rd and 4th days after exposure to radiation. Therefore, this suggests that a hypothetical period of 3–7 days is required before a repeated can be carried out.

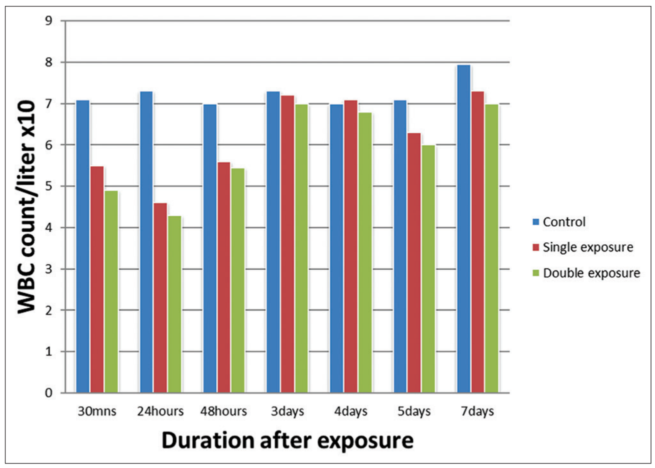

Figure 1: Distribution of postirradiation white blood cell counts in albino Wister rats

Results from the statistical test showed significantly higher WBC and neutrophils counts in the control than in the experimental groups at $P < 0.05$. The concentration of lymphocytes in the experimental groups showed significantly higher count than that obtained from the control group at $P < 0.05$ [Tables 1 and 2].

DISCUSSIONS

The results obtained from this study indicate a significant reduction in leukocyte counts following single and double irradiation of Albino Wister rats. This finding is in agreement with the results of Ikamaise *et al.*[10] and also those of Simovic[11] and Miura *et al.*[12] who conducted similar trials on rats. In experiments on goats and pigs,

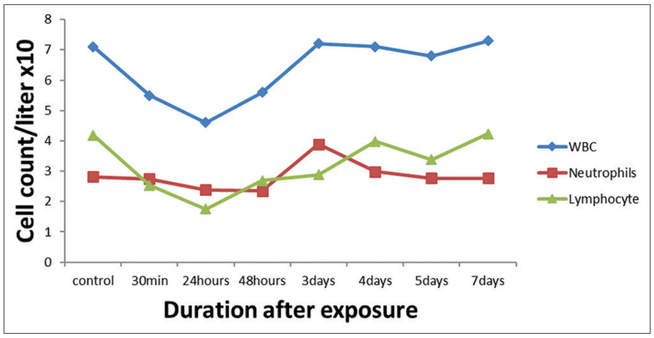

Figure 2: The postirradiation leukocyte recovery curve for single exposure

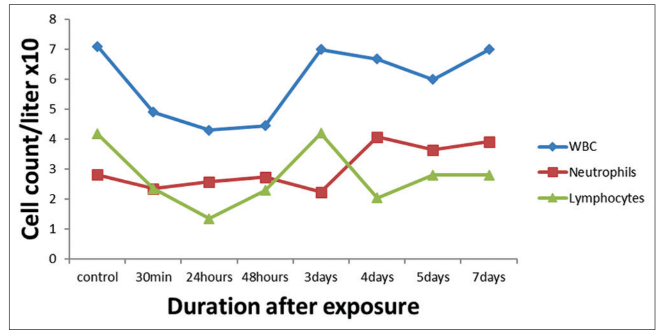

Figure 3: The postirradiation leukocyte recovery curve for double exposure

Milosevic *et al.*[13] demonstrated the same outcome of X-ray irradiation on leukocyte, lymphocyte, and neutrophil granulocyte counts which decreases to 20% of the initial values during the first few days following irradiation. Similar to my findings, the number of these cells in the circulation increased gradually and reached initial values 3–4 days after irradiation. This was a consequence of the X-ray action on the bone marrow progenitor cells and also a consequence of the short-life span of blood cells.[14-17] The effect was more remarkable in the double (repeated)-exposure group than single-exposed group as radiation effect increases with dose[3] thus, a significant difference in leukocyte indices between single exposure and double (repeated) exposure. Although the radiation dose employed in this study was <1 Gy and may be less life-threatening Rozgaj *et al.*[22] reported that long-term exposure to low doses of ionizing radiation may affect the cells and tissues and result in the blood count drops soon after irradiation and recovers within several weeks. Similar studies conducted by Trall *et al.*[23] demonstrated significant reduction in WBC counts at 24 h postirradiation for animals using various dose levels except the 0.25 Gy group. Similarly, Mark *et al.*[24] demonstrated a dose-dependent decrease in WBC counts in mice exposed at doses up to 2 Gy at high (0.5 Gy/min) and low (0.5 Gy/h) dose rates using γ rays and 70 MeV/n protons. On the other hand, these results differ from some of the previously published data by Gridley *et al.*[25] and Pecaut *et al.*[26] who evaluated the dose-rate effect on WBCs by irradiating mice in the dose range of 1–3 Gy with 250 MeV/n protons at dose rates of 0.01 Gy/min and 0.8 Gy/min and did not observe a significant dose-rate effect on WBCs 4 days after the radiation exposure. This result was comparable with that of Alexandr *et al.*,[18] who investigated hematopoietic Recovery after 10-Gy Acute Total Body Radiation in 34-year-old male operator of a 6oCo y-radiation sterilization facility in Nieshvesh. The authors demonstrated that the number of granulocyte decreases at that level of irradiation for a certain period and but were detectable and exceeding 0.5×10^9/L by day 37. Thus, a partial hematologic recovery was recorded until the demise of the patient on day 113. This shows that despite the dosage, there was some level of recovery. Similarly, El-Shanshoury *et al.*[27] also recorded some level of recovery after a statistical decease in the WBC using a dose of 0.3 Gy up to 1 Gy.

Studies have revealed that there is decrease in total WBCs count after irradiation, and hence, WBC count is used as an indicator to exposure.[7,19] The WBC and differential count were comparable in all members of the control group throughout the duration of the study. This observation confirms the homologous status of the rats used in the study. There was a sharp fall in WBC count 24 h postirradiation as shown in Table 1 and Figures 1, 2. The difference was significant at $P < 0.05$. The WBC count began to increase again after 24 h as was observed due to the setting in of recovery mechanism which according to Yamonenko[7] may take place in two ways; repair at the cellular level where sublethally damaged cells recover their viability and by proliferation of undamaged cell elements. The recovery was delayed for lymphocytes till the 48 h in single exposure and 3 days in double (repeated) exposure. This delay in the double-exposed group was as a result of increase in radiation dose.[3]

The process of repair and recovery was gradual and took a longer time than the rate of destruction. The effect of radiation on lymphocytes was exclusively fast because of their high sensitivity to radiation.[8] However, repair and recovery process in neutrophil commenced earlier, and the count in the control group were significantly higher ($P < 0.05$) than in the experimental group (both single and double exposure). The increase in the count of lymphocytes and monocytes which was significantly higher in the experimental group than in the control may be explained by the natural functions of these cells in body defense.[20] During this period of declining bone marrow cells, individuals are at an increased risk of infection and hemorrhage.[5]

All counts showed maximum repair and recovery between the 3rd and the 4th days after exposure to radiation. The maximum repair and recovery recorded was 98.6% for single exposure and 95.9% for double (repeated) exposure of the normal count. This implies that 1.5% of damage done for single exposure group and 4.1% for double (repeated) exposure group could not be repaired within the period of the study. This is in agreement with Frankel[21] who reported the incomplete nature of repair and recovery of blood cells after exposure to radiation. This study however records a maximum cell recovery of 98.6% for single exposure group similar to 98.5% reported by Ikamaise *et al*.[10] and against 90% reported by Frankel.[21] This can be compared with the report of El-Shanshoury *et al*.,[27] who calculated a net recovery of 45% from a dose of 0.3 Gy up to 1 Gy.

The clinical implication of this finding is that for any effect caused by a given quantity of radiation (within diagnostic range), a more pronounce effect will be produce with double (repeated) irradiation. Thus, a minimum of 4 days should be observed before carrying out a repeat except where justifiable and feasible.

CONCLUSION

This study attempted to determine the time for full recovery of the WBCs following single and double (repeated radiation exposure) and to hypothesize a safe period for repeated irradiation for patients undergoing periodic radiographic examinations for follow-up cases. Although the experiment was based on whole body irradiation of the rats, the radiographic factors used were within diagnostic range. This study has presented that a maximum of 3–7 days is required for full recovery of peripheral WBCs after irradiation (within this diagnostic range) and the recovery is not 100%. This will serve as a guide to radiographers/radiologist in the management of patients who need follow-up examinations. A safe period of at least 3–7 days must be allowed for the cells to recover from the previous irradiation before a case is repeated.

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Conflicts of interest

There are no conflicts of interest.

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