POST IRRADIATION CELL RECOVERY IN RATS

Ikamaise V.C.[1], Chiaganam N.O.[1], Dike E.U[2]., Ndem B.N.[3]

1. Department of Radiography, Faculty of Allied Medical Sciences, College of Medical Sciences, University of Calabar, Calabar, Nigeria
2. Radiology Department, National Hospital, Abuja, Nigeria
3. Hematology Department, University of Calabar Teaching Hospital, Calabar, Nigeria.

ABSTRACT

Objective: Post irradiation cell recovery in peripheral white blood corpuscles after whole body x- irradiation was investigated using Albino Wister rats. The study was aimed at the determination of the time for full recovery of the white blood cells in order to hypothesize a safe period for repeated irradiation for patients undergoing periodic radiographic examinations for follow up cases.

Methods: Seven (7) rats were irradiated with X-rays from a diagnostic x-ray machine and blood samples taken at intervals through a period of 30 minutes to nine (9) days. Samples were simultaneously taken from seven (7) other homologous rats serving as control. Haematologic analysis was carried out on the blood samples.

Results: Results from the WBC counts showed a sharp decrease in the first 12 hours after irradiation ($p<0.05$). Recovery of the cells was observed after 24 hours except for lymphocytes. The recovery rate was slower than that of destruction. All counts showed maximum repair and recovery between the 7th and the 8th days after irradiation. The maximum repair and recovery obtained was 98.5% of the normal count.

Conclusion: The implication of this is that there is an irreparable fraction of the damage done by radiation. It follows that for a given amount of radiation dose received there is an effect at first exposure and that a lesser dose at second and subsequent exposures will be required to cause the same effect.

Keywords: Irradiation; blood cell; Recovery; Rats.

Corresponding Author:
IKAMAISE V. C.
Mailing Address: Department of Radiography, Faculty of Allied Health Sciences, College of Medical Sciences, University of Calabar, Calabar.
E-mail: vikamise@yahoo.com
Phone: +234 803573 1144

INTRODUCTION

Biological effects of irradiation have attracted the attention of researchers for decades. X-radiation was discovered on the 8th November 1895 and in March 1896, four months after Roentgen's discovery, adverse effects of x-rays were recorded. It was observed that all animal and human tissues are sensitive to radiation and absorption of radiation doses above certain limits will induce some physiological changes in the tissues.[1] Medically, in the diagnosis and treatment of illnesses, x-radiation is used worldwide. However, successes recorded in medical diagnosis and treatment from the use of x-radiation oftentimes become contentious due to genetic and haemopoietic effects occasioned by its usage.[2]

Although application of radiation involves a certain level of risk, the use of radiation in medicine results in such numerous benefits that if judiciously employed, the benefits greatly exceed the very risk to the individual.[3] The biological effects due to radiation may manifest in clinical symptoms. The nature and severity of these symptoms and the time at which they appear depend on the amount of radiation absorbed and the rate at which it was received.[4] A severe depression of haematopoietic function often takes place in patients undergoing radiotherapy due to the high sensitivity of haematopoietic system to radiation.[5] Martin and Harbison observed that the early effects of radiation are due to cell killing and the prevention or delay of cell division.[6] Henry pointed out that a decrease in lymphocytes count in the peripheral blood is one of the most sensitive tests of radiation exposure.[7] He observed that there is always a slight decrease in the total white cell count after the first few days. Hence, white blood cell count is an indicator of degree of exposure.

Therefore, in the present study we investigated post irradiation cell recovery in peripheral white blood corpuscles after whole body x- irradiation in order to determine time of full recovery and to hypothesize a safe period in between irradiation for people undergoing periodic radiographic examinations for check up or follow up cases.

MATERIALS AND METHOD

Fourteen Wister Albino rats weighing between 200-270g were obtained from University of Calabar Pharmacology Department animal house. They were grouped into two (2) of seven (7) rats

each. One group served as the control (C) while the other formed the experimental group (E). Members of experimental group were irradiated with X-rays using the following exposure factors; 70kV, 75mA, 0.4sec, which is within the diagnostic range from a MX4 X-ray machine manufactured by Watson Electromedical Ltd. The Focus Film Distance (FFD) was 90m. Some haematologic parameters were analyzed for both groups.

BLOOD SAMPLE COLLECTION

A venopuncture was performed on the animals under chloroform anaesthesia to obtain 2ml of blood sample which was transferred to a specimen bottle containing 0.02ml EDTA anticoagulant. The blood samples were obtained at intervals of 30mins 24hours, 48hours, 5days, 7days, 8days and 9days after irradiation for members of the experimental group. Similarly, blood samples were obtained from members of the control group at the same intervals for analysis.

WHITE BLOOD CELL COUNT

Bulk dilution method of white blood cell count was employed. 0.02ml of well mixed EDTA anticoagulated blood was pipetted into 0.38ml of Turks solution contained in khan tube. It was well mixed. A clean cover slip was put in place on the improved Neubauer counter. Using a capillary tube held at an angle of 45° to the counting chamber, the diluted blood sample was carefully discharged unto the counting chamber. The chamber was then placed in a petri dish and left undisturbed for 2 minutes, allowing the cells to settle. The underside of the chamber was dried and placed on the X10 objective of the microscope. The WBCs in the chamber were focused. The cells in the four large corners were counted, including cells on the lines of two sides of the large squares. The number of white cells (per litre of blood) was presented using a correction factor of x 10.[9(6)]

DIFFERENTIAL WHITE CELL COUNT

Longitudinal method of differential white cells count was adopted. A drop of blood was pipetted unto a clean dry microscope slide and a thin film was made. The film was dried in air. The blood film was flooded with Leinshman stain and allowed to stand for ten minutes to achieve complete differentiation. It was then cleaned with water and allowed to dry in air. A drop of immersion oil was placed on the film and covered with a clean dry cover slip and again allowed to dry. The film was viewed under the X40 objective of the microscope. The different white cells seen in each field was counted using the automatic differential cell counter.[7]

STATISTICAL ANALYSIS

Student t-test was applied to compare the data obtained for WBC and differential cell count for significant difference within and between control group and the experimental. Obtained data was also used to plot Post irradiation cell recovery curve.

RESULTS

The WBC and differential count for all members of the control group is presented in table 1. The WBC and differential count was comparable throughout the duration of the study. The dominance of lymphocytes over neutrophils in the control group was observed.

TABLE 1

Total WBC and the Differential Count in Members of the Control Group (C-group)

Sample	Blood Cells	Blood Count/ Litre (10^9)	Per-centage
C_1	Total WBC	8.80	100
	Neutrophil	3.17	61
	Lymphocytes	5.37	36
	Monocytes	0.26	03
C_2	Total WBC	8.60	100
	Neutrophil	3.44	40
	Lymphocytes	5.07	59
	Monocytes	0.09	01
C_3	Total WBC	8.60	100
	Neutrophil	3.10	36
	Lymphocytes	5.25	61
	Monocytes	0.26	03
C_4	Total WBC	8.65	100
	Neutrophil	3.10	36
	Lymphocytes	5.20	61
	Monocytes	0.26	03
C_5	Total WBC	8.60	100
	Neutrophil	3.15	36
	Lymphocytes	5.10	61
	Monocytes	0.26	03
C_6	Total WBC	8.66	100
	Neutrophil	3.20	36
	Lymphocytes	5.30	61
	Monocytes	0.26	03
C_7	Total WBC	8.60	100
	Neutrophil	3.25	36
	Lymphocytes	5.25	61
	Monocytes	0.26	03

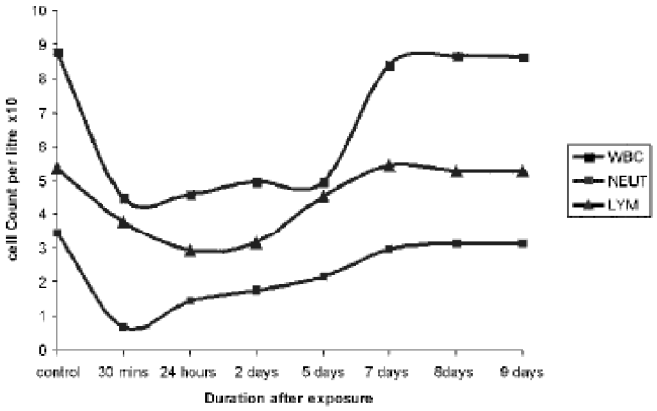
Table 2 presents the WBC and differential count for members of the experimental group. A sharp fall in WBC from 8.80 count/litre to 4.50 count/litre was observed 30 minutes following irradiation. This fall persisted till the 5th day after irradiation. The WBC count returned to normal on the 7th day following irradiation. Neutrophil count was also observed to drop from 3.17 count/litre to 0.68 count/litre in the first 30 minutes after irradiation. The fall in neutrophil count persisted till the 7th day. The lymphocyte count dropped sharply within the first 24 minutes and quickly was restored. Eosinophils and monocytes concentrations also showed sharp decrease in the first 2 days after which they regained gradually.

TABLE 2

Total WBC and the Differential Count in Members of the Control Group (E-group)

Sample	Blood Cells	Blood Count/ Litre (10^9)	Per-centage	Duration after radiatn
E_1	Total WBC	4.50	100	
	Neutrophil	0.68	15	30
	Lymphocytes	3.78	85	minutes
	Monocytes	0 05	01	
E_2	Total WBC	1 43	100	
	Neutrophil	4 60	31	24
	Lymphocytes	2 90	63	minutes
	Monocytes	0 28	06	
E_3	Total WBC	5 00	100	
	Neutrophil	2 14	35	2 days
	Lymphocytes	3.15	63	
	Monocytes	0.05	01	
	Eosinophil	0.05	01	
E_4	Total WBC	5 00	100	
	Neutrophil	2.14	32	5 days
	Lymphocytes	4 56	68	
E_5	Total WBC	8.40	100	
	Neutrophil	2 94	35	7 days
	Lymphocytes	5 46	65	
E_6	Total WBC	3.12	100	
	Neutrophil	5.28	36	8 days
	Lymphocytes	0.09	61	
	Eosinophil	0.17	01	
	Monocytes	8.66	02	
E_7	Total WBC	3 11	100	
	Neutrophil	5 27	36	9 days
	Lymphocytes	0.09	61	
	Eosinophil	0 17	01	
	Monocytes	8.64	02	

Figure 1. demonstrates the post irradiation cell recovery curve. The curve showed rapid destruction of WBC generally and neutrophil specifically and a gradual recovery process. The destruction of lymphocytes was gradual and a faster recovery process was observed. Generally, the three curves (for WBC, neutrophils and lymphocytes) show maximum repair and recovery between 7th and 8th days after exposure to radiation. The maximum repair and recovery obtained was 98.5% of the normal count.



Results from the statistical test showed significantly higher WBC and neutrophil counts in the control than in the experimental group at $p<0.05$. The concentration of lymphocytes and monocytes in the experimental group showed significantly higher count than obtained from the control group at $p<0.05$ (Tables 1 & 2).

DISCUSSIONS

Blood consist of a pale, straw-coloured fluid (the plasma) in which the formed elements (red cells, white cells and platelets) are suspended. The red cells are concerned with transportation of oxygen, white cells are involved in reaction to infections and platelets are involved in the prevention of loss of blood. When blood is exposed to radiation depending on the degree and rate of exposure some cells may die and this will lead to reduction in the cell count. Reduction in the cell count will affect the functions performed by the particular cell. Studies have revealed that there is decrease in total white blood cells count after irradiation and hence WBC count is used as an indicator to exposure.[2,7]

The WBC and differential count was 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.

The observation that differential count showed lymphocytes dominant over neutrophils agrees with the reports of Wagner et al[10] and Weisbroth et al[11] on the blood of laboratory animal as compared to human blood (Table 1).

WBC count for the experimental group showed a sharp decrease in the first 12 hours after irradiation as shown in Table 2 and figure 1. The difference was significant at $p<0.05$. This decrease in count is attributed to the early somatic radiation effect on the WBC.[6] The WBC count began to increase again after 24 hours as was observed due to the setting in of recovery mechanism which according to Yarmonenko[(2)] may take place in two ways; repair at the cellular level where sub-lethally damaged cells recover their viability and by proliferation of undamaged cell elements. The recovery was delayed till after 2 days for lymphocyte. The process of repair and recovery was gradual and took a longer time than the rate of destruction. The effect of radiation on neutrophil was exclusively fast. However repair and recovery process in neutrophil commenced earlier and the count in the control group was significantly higher than in the experimental group at $p<0.05$. 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.[12] All counts showed maximum repair and recovery between the 7th and the 8th days after exposure to radiation. The maximum repair and recovery recorded was 98.5% of the normal count. It therefore follows that 1.5% of damage done cannot be repaired. This is in agreement with Frankel's report on the incomplete nature of repair and recovery of blood cells after exposure to radiation. This study however records a maximum cell recovery of 98.5% as against 90% recorded by Frankel.[3]

The practical implication of this finding is that for a given quantity of radiation received by patients as absorbed dose required to cause a specific effect at first exposure; a lesser dose at second and subsequent exposures will be required to cause the same effect.

CONCLUSION

This study attempted to determine the time for full recovery of the white blood cells to hypothesize a safe period for repeated irradiation for patients

undergoing periodic radiographic examinations for follow up cases. Though 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 8 days is required for full recovery of peripheral white blood cells after irradiation and the recovery is not 100%. This will serve as a guide to radiographers in the management of patients who need follow up examinations. A safe period of at least 8 days must be allowed for the cells to recover from previous irradiation before a case is repeated.Table 1: Total WBC and the Differential Count in members of the control group (C-group).

REFERENCES

1. Jacobi C. A. and Paris D. A. (1977). Textbook of radiographic technology (6th edition). Saint Loius: the C. V. Mosby company; 110-117.
2. Yamonenko S. P (1988); Radiology of human and animals (English edition). Mir Publishers. Moscow, 126 &140.
3. Frankel Robert (1996). Radiation protection for radiation technologist: (2nd edition). McGraw-Hill Book Company, New York.;18-30.
4. Maniatis A., Tavasseli M. and Crosby W. H. (1971). Origin of osteogenic precursor cells in extramedullary marrow implants. Blood, The journal of hematology Vol. XXXVIII, No. 5, 569 – 575.
5. Pointer RCS (1991). The radiotherapy of malignant disease. Springer-Verlag. 68, 111-129.
6. Martin A. and Harbison S. A. (1979). An introduction to radiation protection (2nd edition). Chapman and Hall, London; 41-44.
7. Henry F. (1969). Fundamentals of radiation protection. Wiley intersciences, New York; 111-117.
8. Baker FJ, Silverton RE, (1973): Introduction to medical laboratory technology, (5th edition), Buterworths, London, 572.
9. Lewis SM, Bain BJ, Bates I (2001): Practical Haematology, (9th edition), Church hill livingstone. 26.
10. Weagner J. E. and Manning P. J. (1976). The biology of the guinea pig. New York: Academic press. 67.
11. Weisbrith S. H.; Flatt R. E. and Krans A. L. (1974): The biology of the laboratory rabbit New York; Academic press. 65.
12 Bell, G.H.; Emslie-Smith, D. and Paterson, Colin R. (1980): Textbook of Physiology (10tl Edition), Churchill Livingstone, London & Nev York, 104-118.