ORIGINAL ARTICLE

Radio Protective Role of Growth Hormone on Secondary Ossification Center of Growing Long Bone in Young Albino Rats

Abdul Latif Panwhar[*], *Hemant Kumar*[**], *Muhammad Rahib Jamali*[***]

ABSTRACT

Objective: To evaluate possible effects of Growth hormone in preventing the harmful effects of radiation on secondary ossification centers of growing long bones of young albino rats.

Study Design: Experimental study.

Place & Duration: Experimental work was completed in Six months duration in the department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC) Karachi.

Material & Methods: 30 litters of 10 days age of Albino rats were taken for this study. They were divided into three groups: Group A (Control), Group B was given 5Gy gamma radiation and group C was given radiation and injection somatotrophin(Growth hormone). Each group was further subdivided into two subgroups according to their respective time period of treatment i-e., 2 and 4 weeks respectively. At the end of their respective period of study animals were anasthetised by ether. Dissection was done and their long bones i,e., humerus and femur were taken out and transferred, fixed, processed and embedded in paraffin . 5um thick longitudinal sections were cut with rotatory microtome. The tissues were stained with Alcian blue- Haematoxylin and Eosin stain for measurement of secondary ossification centers.

Results: A highly significant decrease in mean secondary ossification centers was noted in irradiated subgroups as compared with control. And highly significant increase of thickness in secondary ossification centers was noted in growth hormone treated subgroups as compared to irradiated subgroups.

Conclusion: Irradiation causes destruction and reduction in secondary ossification centers. Growth hormone reverses the damage.

Key Words: Long bones, Secondary ossification centers, Irradiation, Growth hormone.

INTRODUCTION

Radiation therapy is a component of curative therapy for a number of malignant diseases. X-rays and gamma rays are the form of radiation most commonly used to treat cancer.

* *Associate Professor, Anatomy Department PUMHS, Nawabshah*

** *Assocate Professor & Head of Anatomy Department Hamdarad Medical University, Karachi*

*** *Demonstrator, Anatomy Department PUMHS, Nawabshah*

Correspondence to:

Dr. Abdul Latif Panhwar
Associate Professor,
Depatment of Aatomy
Peoples University of Medical & Health Science,
Nawabshah.
Cell: 0334-3394804

Radiation is a physical form of treatment that damages any tissue in its path, which causes breaks in DNA and generates free radicals from cell water that may damage cell membranes, proteins and organelles[1]. Dose limiting complications of radiotherapy include skeletal abnormalities and disturbances in skeletal development within the irradiated field[2]. The developing fetus and young children are highly sensitive to growth and developmental abnormalities induced by ionizing radiation[3]. Ionizing radiation affects all phases of physeal activity, but especially chondrocytes and small blood vessels. Radiation damage to these blood vessels results in the irregular production of osteoid and faulty bone formation[4]. Normal tissue damage is the main dose limiting factor in clinical radiotherapy[5,6]. Radiobiological studies have identified several radioprotective compounds some

of which are non-toxic to humans [7]. Growth hormone is an anabolic hormone with effects on growth, differentiation and metabolism of cells[8]. There is currently substantial interest in growth hormone as a protective agent against radiation related normal tissue injury [8,9].

The present study was designed to study the effects of Somatotrophin (growth hormone) on secondary ossification centers, in irradiated long bones of young albino rats.

MATERIAL & METHODS

This experimental study was conducted at Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC) Karachi. 30 newborn litters of Albino rats were obtained from Animal house BMSI, JPMC Karachi. The animals (litters) were weighed and marked on 1st post natal day and divided into 3 groups, i-e, A, B, C each comprising of 10 animals. Each group was further divided into two subgroups, i-e, A1and A2; B1 and B2; and C1and C2 according to their respective time period of treatment, i-e, 2 and 4 weeks respectively. Each subgroup comprised of 5 animals, and was kept in separate cages along with mothers for milk feeding. The mothers were given laboratory feed and water ad libitum. Animals were kept in experimental room for 10 days prior to commencement of study, for acclimatization to the experimental conditions with 12 hours light and dark cycle. Animals were watched daily for their health status. On 10th post natal day animals (litters) were weighed, treated and allowed to survive for their respective period of study.

In the present study animals were treated as under:

Group-A (A1 and A2), animals served as control.

Group- B(B1 and B2), animals received irradiation at the dose of 5 Gy for 2.02 min. from 60-unit cobalt chamber[10,11], at the Department of Radiotherapy JPMC Karachi, at the commencement of study.

Group- C (C1 and C2), animals received Radiation and injection Somatotrophin with a dose of 0.3µg/gm body weight[12]for their respective period of study.

After treatment, all the animals were watched daily for their health status on the basis of their activity and weight gain or loss. On completion of their respective period of treatment animals were sacrificed under ether anaesthesia. Dissection was done and long bones humerus and femur were taken out, transferred and fixed in 10% formalin fixative for 48 hours, then were kept in 5% formic acid for 24 hours and then were processed and embedded in paraffin. The bones were oriented horizontally for longitudinal sections. 5um thick longitudinal sections were cut with rotatory mic-/rotome. Sections were floated on the surface of warm water at 42°C in water bath. Then tissues were transferred on albuminized glass slides, the slides were put on hot plate overnight at 37-40°C for fixation of tissues.

The tissues were stained with, Alcian blue-Haematoxylin and Eosin to observe the regularity of cartilage columns , trabecular network & blood vessels under 8X ocular and 10X objective of light microscope. The sections were observed for measuring the thickness of secondary ossification centers with the help of ocular micrometer scale. 10 areas were randomly selected from each growth plate.

The statistical significance of the difference of various quantitative changes between the experimental and control groups was evaluated by student "t" test. All the calculations were done by utilizing computer software SPSS (Special Package for Social Science) version 10, through Microsoft Excel in Window 2000xp.

RESULTS

The microscopic changes were observed in 5µm thick Alcian blue-Haematoxylin and Eosin, stained sections. The following observations and results were recorded for statistical analysis. Secondary ossification center was observed in both ends of bone, which was filled with trabecular network, and measured (Figure: 01 & 02).

Humerus and femur bones in both subgroups B1 and B2, the structural changes were observed in secondary ossification center, which was reduced in size, and filled with reduced and scattered trabecular network. The mean secondary ossification center extent in the Humerus and femur

of subgroup B1 and B2 showed a highly significant (P<0.001) decrease in diameter of both subgroups as compared to control A1 and A2 respectively (Table-1).

The mean secondary ossification center extent in the humerus and femur of subgroup C1 and C2 showed a highly significant (P<0.001) increase in diameter of both subgroups C1 and C2 as compared to irradiated subgroups B1 and B2 respectively (Table-1). There was highly significant (P<0.001) increase in secondary ossification center extent of humerus and highly significant (P<0.001) decrease in ossification center extent of femur of both subgroups C1and C2, as compared with control subgroups A1and A2 respectively(Table-1).

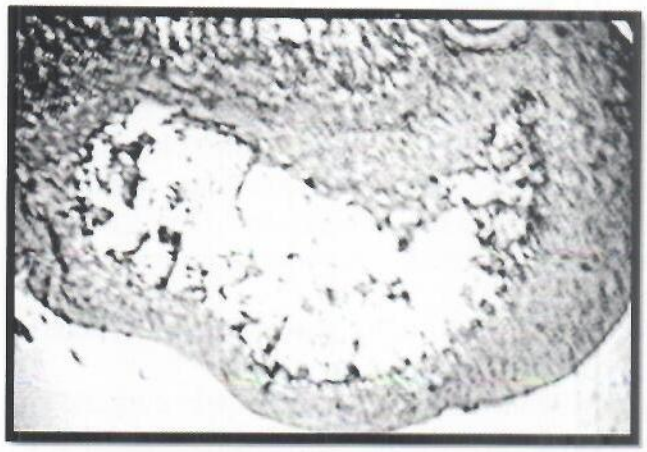

Figure No 01. Effect of Radiation on Secondary Center of Humerus Bone

Figure No 02. Radio Protective Role of Growth Hormone on Secondary Ossification Center of Humerus Bone.

DISCUSSION

Radiations are used in medical treatment and diagnostic procedures. Radiation therapy can be used in combination with surgery and/or chemotherapy to provide permanent control or death of a tumor[11]. In radiation therapy high energy rays are used. The gamma radiation produces anatomical & pathological alterations in bone growth. Several investigators have used experimental gamma radiations in animals. Nunia et al[13], had used Swiss albino mice for whole body gamma irradiation.

Growth hormone can stimulate growth of different tissues, such as skeletal and soft tissues, by increasing number of cells[9]. In the present study in irradiated group animals, the secondary ossification center of bones was reduced in size and myeloid elements decreased. Parker and Berry[14], reported that radiation induces suppression of bone marrow function. In group C secondary ossification center was normal, and filled with myeloid elements as in control group. Matsouka et al[12], reported that low dose irradiation on femur of wistar rats, is capable of inducing apoptosis in bone marrow cells , the growth hormone administration reverse this process. It has been reported that growth hormone directly stimulate the division and multiplication of chondrocytes of cartilage[15].

CONCLUSION:

In the light of above considerations the net result suggest that injurious effect of radiation occur more frequently at a dose of 5 Gy in growing bones of young Albino rats. Irradiation can cause cellular damage, but the Growth hormone, restores the growth. The present study suggest that adverse effects of irradiation need special cautions for human subjects and the study may act as a base line for the extension of project for humans.

Table-1 Mean Extent of Secondary Ossification Center (μm) of Long Bones in different Groups of Albino Rats at Variable Time Intervals

Groups	Subgroups	Treatment given	2 Weeks		4 Weeks	
			Humerus	Femur	Humerus	Femur
A	A1(n=5)	Control	1458.6±3.81	1428.7±6.80	----	---
	A2(n=5)		---	---	1742.6±2.71	1702.5±20.48
B	B1(n=5)	Radiation	536.9±2.72	572±30.71	---	---
	B2(n=5)		---	----	935.7±3.16	901.15±20.70
C	C1(n=5)	Radiation+ Growth Hormone	1723.1±2.08	1763.45±15.89	---	---
	C2(n=5)		----	---	1436.4±6.17	1472.5±7.79

*Mean±SEM±

Table-2 Statistical Analysis of Differences in mean Thickness of Secondary Ossification Center of Long Bones in Different Groups at Variable Time Interval

Statistical comparison	Humerus P-value	Femur P-value	Statistical comparison	Humerus P-value	Femur P-value
B1vsA1	P<0.001****	P<0.001****	B2vsA2	P<0.01****	P<0.001****
C1vsB1	P<0.001****	P<0.001****	C2vsB2	P<0.001****	P<0.001****
C1vsA1	P<0.001****	P<0.001****	C2vsA2	P<0.001****	P<0.001****

Key:*non-significant. **significant. ***moderately significant. ****highly significant

REFERENCES:

1. Fauci A, Braun WE, Kasper D, Hauser S, Longo D, Jameson L, et al. Neoplastic disorders in: Harrisons Principles of International Medicine volume I, 17th edition, New York Chicago, Mc Graw Hill. 2008;479-623.
2. Kumar V, Abbas A, Fausto N. Environmental and Nutritional Pathology in: Robbins and Kotran Pathologic Basis of Disease. 7th edition, Philadelphia Pennsylvania, Saunders Elsevier; 2004;436-9.
3. Pateder DB, Eliseev RA, O'Keefe RJ, Schwarz EM, Okunieff F, Constine LS, et al. Role of autocrine growth factors in radiation damage to epiphyseal growth plate. Radiat Res. 2001;155(6):847-57.
4. Essman SC, Lattice J, Cook JL, Turnquist S, Kuroki K. Effect of Ethylene diamine tetramethylene phosphonate on physeal and articular cartilage in Juvenile Rabbits. J Nuclear Med.2003;44(9):1510-5.
5. Akyurek S, Atahan L, Cengiz M, Sokmensuer C, Haberal I, Yildiz F, et al. Effect of ticlopidine in the prevention of radiation enteropathy. Br J Radiol.2006; 79:409-14.
6. Eiffel PJ, Donaldson SS, Thomas PF. Response of growing bone to irradiation: a

proposed late effects scoring system. Int J Radiat Oncol Biol Phys. 1995;31:1301-7.
7. Prasad KN, Cole WC, Hasse GM. Radiation Protection In Humans: Extending the concept of as low as achievable from dose to biological damage. Br J Radiol. 2004;77: 97-9.
8. Madrid O, Varea S, Peraz IS, Gomez-Garcia L, Miguel ED, Segura G, et al. Growth hormone protects against radiotherapy-induced cell death. Eur J Endocrinol.2002; 147:535-41.
9. Tekin SB, Ertekin MV, Erdogon F, Sezen O, Karslioglu I, Gepdiremen A, et al. Is growth hormone a radioprotective agent? J Eur Acad Dermatol Venereol.2006;20(3):293-8.
10. Koc M, Tayasi S, Buyukokuroglu ME, Bakan N. Melatonin protects rat liver against irradiation-induced oxidative injury. J Radiat Res.2003;44(3):211-5.
11. Nash H. Radiation therapy in Dogs, Cats, and other small animals. Veterinary Services Department.2008;1-4.
12. Matsouka P, Mylonas P, Papandoniou E, Dimitropoulou I, Floratou K, Alexandridis T, et al. Abdominal radiation initiates apoptotic mechanism in rat femur bone marrow cells in vivo that is reversed by IGF-1 administration. J Radiat Res. 2008;49(1):41-7.
13. Nunia V, Sancheti G, Goyal PK. Protection of Swiss albino mice against whole-body gamma irradiation by diltiazem. Br J Radiol. 2007;80:77-84.
14. Parker RG, Berry HC. Late effects of therapeutic irradiation on the skeleton and bone marrow. Cancer. 1976;37(2):1162-71.
15. Binder G, Wittekindt N, Ranke MB. Noonan Syndrome: genetics and responsiveness to growth hormone therapy. Horm Res. 2007;67(1):45-9.