Original Article

Radiation and Growth Hormone

Radioprotective Effect of Growth Hormone (Somatotrophin) on Irradiated Long Bones of Young Albino Rats

1. Abdul Latif Panwhar 2. Hemant Kumar 3. Naheed Gohar 4. Abdul Ghafar Ansari

1. Asstt. Prof. of Anatomy, PUMHSW, Nawabshah 2. Assoc. Prof. of Anatomy, Islam Medical College Sialkot 3. Asstt. Prof. of Anatomy, Sir Syed College of Medical Sciences for Girls, Karachi 4. Prof. of Anatomy, PUMHSW, Nawabshah

ABSTRACT

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

Study Design: Experimental study

Place and Duration of Study: This study was carried out at Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC) Karachi from January 2009 to June 2009.

Materials and Methods: 30 litters of 10 days of age of albino rats were taken for this study. They were divided into three groups: Group A (Control),Group B(Irradiated) was given 5Gy gamma radiation and Group C was given radiation and injection somatotrophin(Growth hormone). Each Group was further subdivided into two sub Groups according to their respective time period of treatment i-e., 2 and 4 weeks respectively. Their weight was observed weekly. Animals were sacrificed by etheranasthesia. Dissection was done and the long bones i,e., humerus and femur were taken out and their length and width measured by digital caliper.

Results: A significant decrease in weight was noted and there was significant decrease in length and width of long bones in irradiated sub Groups B as compared to control Group A. There was significant increase in weight, length and width in long bones of irradiated and somatotrophin treated sub Groups of C as compared to irradiated sub Groups of B.

Conclusion: Irradiation causes severe bone growth retardation. Growth hormone protects the bone injury and reverses the damage.

Key Words: Radiation, Growth Hormone, Digital Caliper

INTRODUCTION

Humans are exposed to ionizing radiation from a variety of sources[1]. On the human body its effects vary from local tissue necrosis to genetic damage, cancer and death[2].

Radiation is a potent mutagen and carcinogen; however, it is also used in the diagnosis and treatment of human diseases. The use of radiation in medicine has always been rationalized on basis of risk versus benefit[3]. Radiation therapy plays an important role as part of multi-modality treatment for a number of childhood malignancies. Dose limiting complications of radiotherapy include skeletal abnormalities and disturbances in skeletal development within the irradiated field[4].The developing fetuses and young children are highly sensitive to growth and developmental abnormalities induced by ionizing radiation. In infants and young children exposed to radiation, bone growth and maturation may be retarded [5].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[6].Irradiation of growing bone typically results in retardation of longitudinal growth[7]. Normal tissue damage is the main dose- limiting factor in clinical radiotherapy[8]. Whole body irradiation has been reported to retard growth in man after exposure to irradiation from atomic bomb explosion, and in animals after varying doses of X-ray during fetal life or infancy[9].

Growth hormone is an anabolic hormone with effects on growth, differentiation and metabolism of cells. Treatment with growth hormone reduces radiation-associated mortality[10].Growth hormone stimulates cartilage and bone growth by increased deposition of protein by the chondrocytic and osteogenic cells that cause bone growth, increased rate of reproduction of these cells, and a specific effect of converting chondrocytes into osteogenic cells, thus causing deposition of new bone[11].The Growth hormone is administered with a dose of 0.2- 0.3µg/gm of body weight in animals [12].There is currently substantial interest in growth hormone as a protective agent against radiation related normal tissue injury[10, 13].

The present study was designed to study the effects of Somatotrophin (Growth hormone), in irradiated long bones of young albino rats.

MATERIALS AND 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 and C each comprising of 10 animals. Each group was further divided into two sub Groups, i-e, A1and A2; B1 and B2; C1and C2 according to their respective time period of treatment, i-e, 2 and 4 weeks respectively. Each sub Group comprised of 5 animals, and were 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 were weighed, treated and allowed to survive for their respective period of study.

Group- A (**A1 and A2),** 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 [14, 15], 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 2- 0.3µg/gm of 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, and weighed weekly. On completion of their respective period of treatment animals were sacrificed by giving etheranaesthesia. They were fixed on dissecting board, and left side limbs were disarticulated and fixed in 10% formalin for 72 hours. Long bones Humerus and Femur were taken out and their length and width measured in mm by electronic digital caliper.

The statistical analysis was done 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 animals of group A were looking healthy, active, taking breast feed regularly, hair were evenly distributed on the body. Other gross changes in this group were observed as under:

The body weight of animals in both subgroups A1and A2 was increased during their respective period of time.There was a highly significant P<0.001 weight gain in the final weight of both sub Groups as compared to their initial weight Table-1. The mean length and width of Humerus and femurin sub Group A1 and A2 was recorded (Table-2 and table3).

The animals in both sub Groups B1 and B2 were inactive, looking ill, weak, sluggish movements, not taking breast feed regularly; hair were irregularly distributed on the body.There was a highly significant P<0.001 weight gain in the final weight of both subgroups as compared to their initial weight. But there was a highly significant P<0.001 decrease in body weight in both subgroups as compared to control of same duration (Table-1). The mean length of Humerus showed a moderately significant P<0.01 decrease, and significantP<0.02 decrease in subgroup B1 and B2 respectively as compared to control sub Groups A1 and A2 respectively. The mean length of femur showed a moderately significant P<0.01 decrease, and highly significant P<0.001 decrease in the length in subGroup B1 and B2 as compared to control subGroups A1 and A2 respectively (Table-2).The mean width of Humerus showed a significant P<0.03 decrease in both subgroup B1 and B2 as compared to control subgroups A1 and A2 respectively. The mean width of femur showed a moderately significant P<0.01 decrease in the width sub Group B1 and B2 as compared to control sub Groups A1 and A2 respectively (Table- 3).

The animals in group C initially were weak and inactive but after 1 week they regained weight and became active. At the time of sacrifice they were active, looking healthy; hairs were equally distributed on the body. There was a highly significant P<0.001 weight gain in the final weight of both subgroups as compared to their initial weight. There was a highly significant P<0.001 increase in body weight in C1, and significant P<0.05 increase in sub Group C2 as compared to irradiated sub Groups B1 and B2 respectively. However there was moderately significant P<0.01 decrease in sub Group C1 and highly significant P<0.001 decrease in mean body weight in sub Group C2 when compared to control sub Groups A1 and A2 respectively(Table-1). The mean length of Humerus showed a highly significant P<0.001 increase of length in sub Group C1and there was insignificantP>0.05 decrease in sub Group C2, as compared to irradiated subgroups B1 and B2 respectively. There was insignificant P>0.05 decrease in length of humerus in both sub Groups C1 and C2, when compared with control sub Groups A1 and A2 respectively (Table- 2).The mean length of femur showed a highly significant P<0.001 increase in C1, and there was insignificantP>0.05 increase in the length of femur in sub Group C2 as compared to irradiated subgroups B1 and B2 respectively. There was insignificant P>0.05 increase in length of femur in sub Group C1 and significant P<0.02 decrease in sub Group C2, when compared with control subgroup A1 and A2 respectively (Table-2). The mean width of Humerus showed insignificantP>0.05 increase in both sub Groups C1 and C2, as compared to irradiated sub Groups B1 and B2 respectively (table-3). The mean width of femur showed insignificant P>0.05 increase in subGgroup C1, and insignificant P>0.05 decrease in sub Group C2, when compared with irradiated sub Groups B1 and B2 respectively. There was significant P<0.04 decrease in sub Group C1, and highly significant P<0.001 decrease in sub Group C2, when compared with control sub Groups A1 and A2 respectively (Table- 3).

Table No.1: Mean Body Weight (gm) of Animals in Different Groups at variable time intervals

groups	subgroups	Treatment given	Initial weight	Final weight at sacrificial time	
				2nd week	4th week
A (n=10)	A1	Control	9.177±0.02	23.99±	
	A2		13.88±0.15		41.39±0.62
B (n=10)	B1	Radiation	9.24±0.03	14±0.56	
	B2		10.59±0.14		26.89±0.26
C (n=10)	C1	Radiation + Growth hormone	12.77±0.21	20.81±0.23	
	C2		9.26±0.17		28.78±0.35

Key:*Mean±SEM

Statistical analysis of the difference in mean body weight in same group and in different groups

Statistical comparison	p-value	Statistical comparison	p-value
B1vsA1	<0.001****	B2vsA2	<0.001****
C1vsB1	<0.001***	C2vsB2	<0.05**
C1vsA1	<0.001****	C2vsA2	<0.001****

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

Table No.2: *Mean Length of long bones (mm) in Different Groups at variable Period of Albino rats

Groups with treatment given	Sub group	2weeks		4weeks	
		humerus	femur	humerus	femur
A (Control)	A1(n=5)	12.62± 0.38		--	--
	A2(n=5)	---		15.08± 0.41	18.25± 0.19
B (Radiation)	B1(n=5)	10.59± 0.25	11.21±0.17	---	----
	B2(n=5)	---	---	13.45±0.15	14.39±0.41
C (Radiation + growth hormone)	C1(n=5)	12.83±0.10	14.41±0.10	---	---
	C2(n=5)	---	---	13.11±0.09	15.31±0.67

Key:*Mean±SEM

Statistical analysis of differeces in mean lengths of bones between different groups at variable time interval

Statistical comparison	Humerus p-value	Femur p-value	Statistical comparison	Humerus p-value	Femur p-value
B1vsA1	P<0.01***	P<0.01***	B2vsA2	P<0.02**	P<0.001****
C1vsB1	P<0.001****	P<0.001****	C2vsB2	P>0.05*	P>0.05*
C1vsA1	P>0.05*	P>0.05*	C2vsA2	P<0.01***	P<0.02**

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

Table No. 3: *Mean width of long bones (mm) in Different Groups at variable Period of Albino rats

Groups with treatment given	subgroups	2weeks		4weeks	
		humerus	femur	humerus	femur
A (Control)	A1(n=5)	1.432±0.05	1.82±0.1		
	A2(n=5)			1.57±0.047	2.188±0.04
B (Radiation)	B1(n=5)	1.15±0.006	1.40±0.09		
	B2(n=5)			1.31±0.08	1.796±0.08
C (Radiation +growth hormone)	C1(n=5)	1.406±0.12	1.566±0.004		
	C2(n=5)			1.396±0.03	1.668±0.01

*Mean± SEM

Statistical analysis of differences in mean width of long bones between different groups at variable time interval

Statistical comparison	Humerus p-value	Femur p-value	Statistical comparision	Humerus p-value	Femur p-value
B1vsA1	P<0.03**	P0.01***	B2vsA2	P<0.03**	P<0.01***
C1vsB1	P>0.05*	P>0.05*	C2vsB2	P>0.05*	P>0.05*
C1vsA1	P>0.05*	P<0.04**	C2vsA2	P>0.05*	P<0.01***

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

DISCUSSION

Radiations are used in medical treatment and diagnostic procedures[15].Several investigators have used experimental gamma radiations in animals. Nunia et al, 2007[16], had used Swiss albino mice for whole body gamma irradiation. These animal studies describe the radiation injuries in experimental animals. In this regard many naturally occurring, anti-oxidants exhibit protection against irradiation injuries. The potential of antioxidants to reduce cellular damage induced by ionizing radiation has been studied in animal models, for more than 50 years. Growth hormone is also a radioprotective agent[13]. Growth hormone can stimulate growth of different tissues, such as skeletal and soft tissues, by increasing number of cells[13].In the present study, animals were given gamma radiation at the dose of 5Gy, as the Engstrom, 1997[17] used the same dose in rat tibia. The selection of same dose was adopted, as our experiment was same, and at this level the radiation causes the growth retardation. In the present study animals treated with irradiation in group B, appeared ill looking, inactive with sluggish movements, not taking breast milk and hairs were irregularly distributed on the surface of body. It might be because of injurious effects of radiation, which disturb the gastrointestinal epithelium, because gastrointestinal epithelium is highly susceptible to radiation injuries [5]. The animals of group A, gained the body weight, throughout the experimental period of study. The body weight was lost in the animals of group B, from 2nd to 4th week, because they were not taking interest in breast feed that might be due to destruction of gastrointestinal epithelium and also damaging of other tissues of body. The animals of group C, showed poor weight loss, because these animals were treated with Growth hormone. The growth hormone normalizes the growth throughout the body as reported by Raguso[18]. Growth hormone stimulate growth of different tissues including skeleton and soft tissues[13].Sert et al., 2006[19] reported that growth hormone administration with radiotherapy protected the intestine in rats. It was in agreement of Bakker et al., 2003[20] who observed that radiation resulted in persistent growth delay in tissue of the irradiate tibiae. This is also in agreement with Larue et al., 1987[7], who reported irradiation of long bones typically results in retardation of longitudinal growth.

In the present study width of long bones was measured at different periods. In irradiated group the width was less than the control. It might be due to injurious effect of radiation on cartilage and bone at the diaphysis level[5].In group C, the width of long bones was protected by growth hormoneand width was similar to control. Guyton and Hall, 2006[11], also reported that there are multiple effects of growth hormone on bone, including increased deposition of protein by the chondrocytic and osteogenic cells that cause bone growth, increased rate of reproduction of these cells, and a specific effect of converting chondrocytes into osteogenic cells, thus causing deposition of new bone. Therefore, the bones can continue to become thicker throughout life, under influence of growth hormone. In the light of above considerations the net result suggest that injurious effect of radiation occur more frequently in growing bones of young albino rats. Irradiation can cause cellular damage, but growth hormone restores the growth.

CONCLUSION

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. This study concludes that gamma radiation produces destruction of epiphyseal growth plate in rats, which can be minimized by Growth hormone. The result of present study is considered promising enough to warrant further studies on animals and trial on human subjects.

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Address for Corresponding Author:
Dr. Abdul Latif Panwhar,
Assistant Professor Anatomy,
Peoples University of Medical and Health Sciences for women NawabShah,
Mobile #: 03343394804
Email: dralpanwhar@yahoo.com