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A STUDY OF BONE GROWTH IN DEXAMETHASONE SODIUM PHOSPHATE TREATED MICE

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ABSTRACT

This investigation was carried out to study the effects of glucocorticoid hormone on skeletal tissues Administration of glucocorticoid in experimental animals produced disorganization of proliferation and hypertrophied cells. In the study seventy two immature male and female mice were taken and divided in three groups. Twenty two mice remained untreated and 24 male and female mice were treated upto 6 weeks with daily doses of dexamethasone twenty four similar mice were given injection of vehicle in which dexamethasone was suspend. In the present study it was found that proximal epiphyseal plate of immature mouse is highly sensitive to high doses of glucocorticoid.

Introduction

Corticosteroids play a role in causing loss of skeletal tissue on use for a long period osteoporosis occurs.

The use of corticosteroid in therapy has produced an iatrogenic form of osteoporosis first described by Boland and Headley (1950) and subsequently by other authors. It depend on the type of the steroid, the duration of therapy, dosage and many other factors. According to Sprague osteoporosis occurs in 40% of cases of Cushings syndrome.

There is marked decrease in glucose utilization if steroids are administered for prolonged period (Kornel, 1973). There is an increased breakdown of proteins and lipid concomitant with a decreased synthesis of RNA proteins and lipids (Baxter and Forsham, 1972). There is reduction of collegen and sulphated mucopolysaccharides (Dougherty et al., 1973).

Administration of corticosteroids in experimental animals produce wide spread osteoporosis particularly of trabecular bone associated with increased osteoclastic resorption. Plasma calcium is also lowered.

Studies by Simmons and Kunin (1967) indicated that cortisol also interferes with endochondral ossification. Similar effect of triamcinolone acetate were also observed with significant morphological changes in cartilaginous plate followed by complete cessation of bone growth and severe osteoporosis (Lime and Kedar, 1976).

Prolonged use of corticosteroid leads to reduced growth of bone in children. Systemic use of corticosteroids for prolonged period results in injurious effects (Heimann and Freberger, 1960). Excretion of bone forming minerals may also occur (Eisenberg and Gordan, 1961) and its use has become limited due to osteoporosis it causes (Cope, 1972).

Affection of growth plate:

The plate is sensitive to general body changes, hormonal alterations and lack of nutrients essential for specific metabolic mechanism (Harrison and Harrison, 1961). Malnutrition, starvation, acute and chronic illness deprive the plate of adequate essential protein and other substances necessary for rapid growth. The diminished cellular activity and matrix production in the growth zone will retard the longitudinal growth of bone. Vitamins and essential substances in the body metabolism especially Vitamin C and D. Vitamin C helps in formation of Collagen tissues (Bourne 1956) Pituitary hormones also control growth of the plate (Asling, 1956).

Materials and Methods

Seventy two immature mice (36 females and 36 males) of J.P.M.C. breed weighing between 15 to 17 gm and aged between 4 and 4.5 weeks were taken from the animal house of Jinnah Post Graduate Medical Centre. They were maintained on normal standard diet.

Two days before experiment the animals were marked by cutting their toes.

Grouping of animals:

The animals were divided into three main groups.

GROUP A (NORMAL CONTROL)

This main group of 24 mice (12 females and 12 males) served as control in order to see the various changes in bone growth at intervals of 2, 4 and 6 weeks. These animals were further divided into three sub groups.

Sub group A IF

This group consisted of 4 female mice, they were sacrificed 2 weeks after the commencement of the experiment and served as control for animals of treated and placebo groups.

Sub group A 2F

This group consisted of 4 female mice and were killed 4 weeks after the commencement of the experiment.

Sub group A 3F

This consisted of 4 females mice were killed 6 weeks after the commencement of the experiment.

Sub group A 1M

This consisted of 4 male mice. They were sacrificed 2 weeks after the commencement of experiment and served as control for animals treated as control groups.

Sub group A 2M

This group consisted of 4 female mice, these animals were killed 4 weeks after the commencement of experiment.

Sub group A 3M

This group consisted of 4 mice and were killed 6 weeks after commencement of experiment.

GROUP B TREATED

This group consisted of 24 mice (12 females and 12 males).

The animals were given Dexamethasone sodium phosphate by daily I/m injection. The dose was calculated at 0.16 ug/g of body weight in a volume of 0.12 ml. These animals were further divided into three sub groups, similar to those of control.

Sub group B IF

Four female mice were sacrificed after two weeks of experiment.

Sub group B 2F

Four female animals were sacrificed after four weeks of experiment.

Sub group B 3F

Four female animals were sacrificed after six weeks of experiment.

Sub group B IM

Four male animals were sacrificed after two weeks of experiment.

Sub group B 2M

Four male animals were sacrificed after four weeks of experiment.

Sub group B 3M

Four male animals were sacrificed after six weeks of experiment.

GROUP C PLACEBO

This group consisted of 24 mice (12 females and 12 males) and was given placebo treatment by Um injection of 032 ml of the solvent used for the corticosteroids. Like the other groups this group was further divided into three sub groups.

Sub group C IF

Four female mice were sacrificed after two weeks of experiment.

Sub group C 2F

Four female mice were sacrificed after four weeks of experiment.

Sub group C 3F

Four female mice were sacrificed after six weeks of experiment.

Sub group C 1M

Four male mice were sacrificed after two weeks of experiment.

Sub group C 2M

Four male mice were sacrificed after four weeks of experiment.

Sub group C 3M

Four male mice were sacrificed after six weeks of experiment.

Administration of drug:

Injection of Dexamethasone phosphate MSD (Decadrone) was used each ml containing 4 ml containing 4 mg of the drug in 1 ml, for mouse 0.16 u/g of body weight was administered i.e. about the twice of the human corresponding dose. Amount of drug for 15 Gm of the mouse was = 15 x 0.16 = 2.4 mg.

Placebo contained

Creatinine	8 mg
Sodium citrate	10 mg
Methyl paraben	0.2 mg

Dissolved in 1 ml of solution
0.1 ml of the solution was used.

Removal of Tibia

The tibias were removed and weighed by torsion balance and processed.

Mean width of proximal epiphyseal plate

The mean width of proximal epiphyseal plate of group A1F was 272.11 ± 4.01µm and it was compared with BiF group and it was 193.96 ± 5.57 µm. This value is less than the control group and the difference was statistically significant (P <0.5 table 1).

The mean width of proximal epiphyseal plate of AIM group was 322.26 ± 19.69 µm and it was compared with treated group BIM and it was 181.88 ± 3.21 µm. The difference was statistically insignificant (P<0.05 table 2).

The mean width of group A1F was compared with CIF. The mean width of CIF was 265.88 ± 12.74 µm. The difference was statistically not significant (P> 0.05 table 1).

The mean width of group A1M was compared with C1M, where the mean width of this group was 231.44 ± 6.85 µm, it was statistically significant (P <0.05 table 2).

The mean width of group A2F was 172.0 ± 3.61µm when it was compared with B2F the mean width was 153.25 ± 8.43 µm, the difference was statistically significant (P< 0.05 table 1).

The mean width of group A2M was 168.87 ± 4.60µm where the mean of width of B2M was 142.77 ± 3.15 µm. It was less than the control group the difference was statistically significant (P< 0.05 table 2).

The mean width of A2F was compared with C2F it was 161.28 ± 4.94µm. The difference was statistically not significant (P >0.05 table 1).

The mean width of A2M was compared with mean width of group C2M. It was 179.55 ± 794µm, it was statistically not significant (P >0.05 table 2).

The mean width of proximal epiphyseal plate of group A3F was 169.94 ± 5.6µm and the difference was compared with B3F group and it was 125.63 ± 3.76µm. It was statistically significant (P<0.05 table 1).

The mean width of epiphyseal plate of group A3M was 179.81 ± 5.76µm and it was compared with B3M and it was 110.54 ± 4.29µm, statistically it was significant (P < 0.05 table 2).

The mean epiphyseal width of group A3F was compared with OF and it was 154.4 ± 3.99 µm. It was statistically significant (P <0.05 table 1).

The mean width of group A3M was compared with OM and it was 166.66 ± 7.87µm. It was statistically not significant (P> 0.05 table 2).

The lower epiphyseal plate

The lower epiphyseal plates were also examined and it was seen that 2-3 cell layer existed in some plates while in majority of the cases the lower epiphyseal end was closed, this being a non growing end of the tibial bone.

Cellular arrangement

The proximal epiphyseal plate was markedly affected by the systemic administration of pharmacological doses of dexamethasone throughout experiment. The non treated and placebo treated epiphyseal plates exhibited the four typical zones.

i) the resting zone in which immature chondrocytes were scattered sparingly in the inter-cellular matrix; ii) the zone of proliferating cells which were arranged in long parallel columns and were close together centrally and at periphey they are separated by homogenous eosinophilic matrix; iii) the hypertrophied layer consisted of large maturing cells. This zone may also called the zone of provisional calcif¡cation; iv) the zone of clarifying matrix adjacent to the diaphysis. The chondrocytes were degenerating and the open ends of their enlarged lacunae are being invaded by capillary loops and premature osteogenic cells from the marrow space of diaphysis are seen. The proximal tibial epiphyseal cartilage of placebo exhibited typical chondrocytic column zonation and eosinophiic matrix. The dexamethasone treatment altered appreciably the width and architecture of the proximal tibial epiphysis. The epiphyseal cartilage was significantly narrowed, the collapse of the articular cartilage seen in figures. The decreased in width was the result not only of reduction of cell population in both proliferation and hypertrophic zones but of a concomitant reduction in the size of the individual cells, thus inhibiting its proper calcification. In addition the diaphysis occasionally appeared osteosclerotic with narrowing of marrow space. The trabeculaer were thicker and anastomosed freely.

Discussion

The quantitative observation in the present study indicate the proximal epiphyseal plate of an immature mouse is highly sensitive to high doses of a glucocorticoid hormone. Of special significance is the fact that as few as 15 injections are sufficient to bring about deleterious effects.

From the skeletal point of view the most significant part of the total metabolic effect of cortisone is the prevention of protein formation and the increased rate of protein destruction. This results in a greater availability of amino acids which are converted into

carbohydrates. The administration of cortisone over a prolonged period will also reduce the protein anabolic of the body, so that osteoporosis will be caused. The osteoporosis is in fact a reduction of the total quantity of bone present in the body. This commonly results in fracture of the bone because of its inability to resist against stress. Even the repair of the fracture is inhibited by the reduced protein (collagen) formation and graft incorporation.

In the present study the experimental animals exhibited epiphyseal plate that was significantly narrowed and were characterized by a disorganization of the proliferation and hypertrophied cells. Thus the difference observed in the structural arrangement of the epiphyseal cartilage in the non treated, placebo and the dexamethasone treated animals indicated that the action of dexamethasone on skeletal growth was specific and not due to any other factor. The adrenocortical steroids are known to influence the ground substance of connective tissue.

Schiller and Adorjman (1957) demonstrated in the rat an inhibition of radioactive sulphate incorporation into the mucopolysaccharides of connective tissue under the influence of cortisone. Inhibition of mucopolysaccharide synthesis by cortisone was also found and the restoration of normal rigidity and reaccumulation of the basophilic ground substance of pepsin treated cartilage was largely prevented by cortisone, cortisol and prednisolone.

In 1963 Bernick and Ershoff suggested that chondroitin sulphate plays a specific and important role in calcification by the participation of its acidic group in helping to fix the calcium. The acid mucopolysaccharides exhibit metachromasis with basic aniline dyes such as toluidene blue the metachromatic staining of calcified cartilage disappeared as calcification took place. It is also shown by the same author that immature or poorly calcified bone stained more intensely. In the present study the non treated epiphyseal plate of the control animals when stained with H & E exhibited typical chondrocytic column, zonation, eosinophilic matrix and well developed trabeculae. In contrast the experimental esiphyseal plates revealed a marked change in the cellular arrangement within the longitudinal columns and also there was alteration in the number and thickness of hone trabeculae on both sides of the plates. The matrix became intensely basophilic instead of the usual eosinophilic. Large sized chondrocytes, morphological alteration accompanied by a complete breakthrough, establishing direct communication between the metaphyseal and epiphyseal marrow cavities, marked morphological changes were also found in the adjoining bone, trabeculae. In addition the diaphysis occasionally appeared "osteosclerotic" with narrowing of marrow space, that is, the trabeculae were thicker.

The results of present study confirmed the results of Bernick and Ershoff (1963) that there was an alteration of the ground substance by giving subcutaneous injection of cortisone acetate to adult male rats for 3 weeks, which produced a loss of body weight and extreme narrowing of the epiphyseal cartilage of the tibia. The diaphysis of the bone had an osteosclerotic appearance due to the fusion and the persistance of the primary trabeculae characterized by a side core of calcified cartilage lined by a thin layer of bone. The trabeculae were anastomosed more obviously with each other. There was a loss of marrow spaces the osteosclerotic appearance of bone in this study. Also this study correlated with the study of Foolis (1951), he believed the persistence and the density of the primary trabeculae in the cortisone treated animals were a result of disturbance in osteolytic activity. This may also be the result of an interference with the calcification which would inhibit normal osteoclastic activity.

Several theories have been suggested concerning the mechanism by which glucocorticoids affect the bone and cartilage of long bones. (i) they may stimulate the production of typical megakaryocytes which subsequently secrete abnormal sticky platelets into the systemic circulation. The latter enhance the formation of thrombi which occulde intraosseous nutrient canals (ii) they may induce subchondral micro fracture leading to bone collapse and necrosis (Solomon, 1973), iii) they may affect lipid metabolism directly, resulting in severe hyperlipaemia, hypercholesterolaemia (Kornai, 1973) and fatty liver. Recent studies of Fisher et al. (1972) have paid considerable attention lipid disorders and impairment of bone formation.

From the data presented in this study as well as the observations reported by other workers, one may conclude that dexamethasone by its action upon acid mucopolysaccharides alters the ground substance of bone, thus inhibiting its proper calcification, its physiologic resorption and therefore its reorganization. Cartilage is not considered primary target for glucocorticoids. It has been suggested that the accelerated narrowing of the epiphyseal plate, results from failure of chondrocytes to hypertrophy and mature (Simmons and Kunin, 1967). Such failure could be the result either of subchondral vascular blockage or of a supressive effect of corticosteroids upon the glucose metabolism and glucolytic cycle of chondrocyte (Kunin and Mayer, 1969). Recently, Priest and Blackwood (1974) have shown a marked depletion of glycogen in chondrocytes treated with corticosteroids. This would undoubtedly inhibit ATP production as well as synthesis of collagen lipids and various other macro-molecules. The fact that in the present study chondrocytes were found to form clusters could possibly be related to the inability of these cells to synthesize sulphated glycosomino glycans.

In contrast to the repidity of the plate response, endosteal bone was relatively slow to react to the hormonal treatment. In the present study 45 injections were necessary to

bring about obvious bone pathology while only 15 injections were necessary before changes were seen in the epiphyseal cartilage. However, once bone damage became evident trabecular fragmentation and bony sequestration progressed rapidly.

Table 1
Mean width of proximal epipheseal plate in female mice

Group No.	Sub Groups	No. of animals	No. of epiphyseal Plate	*Mean width of Epiphyseal Plate (μm)	P Value of difference as compared to control group (A)
	AIF	4	8	272.11 ± 4.01	
A	A2F	4	8	172.00 ± 3.61	
	A3F	4	8	169.94 ± 5.66	
	B1F	4	8	193.96 ± 5.57 ($P < 0.05$)	Significant
B	B2F	4	8	153.25 ± 8.43 ($P < 0.05$)	Significant
	B3F	4	8	125.63 ± 3.76 ($P < 0.05$)	Significant
	C1F	4	8	265.88 ± 12.74 ($P > 0.05$)	Not significant
C	C2F	4	8	161.28 ± 4.94 ($P > 0.05$)	Not significant
	C3F	4	8	154.40 ± 3.99 ($P < 0.05$)	Significant

* = S.E. Mean standard error.

Table 2
Mean width of proximal epipheseal plate in male mice

Group No.	Sub Groups	No. of animals	No. of epiphyseal Plate	*Mean width of Epiphyseal Plate in μm	P Value of difference as compared to control group (A)
	A1M	4	8	322.26 ± 19.60	
A	A2M	4	8	168.87 ± 4.60	
	A3M	4	8	179.81 ± 5.76	
	B1M	4	8	181.88 ± 3.21 ($P < 0.05$)	Significant
B	B2M	4	8	142.77 ± 3.15 ($P < 0.05$)	Significant
	B3M	4	8	110.54 ± 4.29 ($P < 0.05$)	Significant
	C1M	4	8	231.44 ± 6.83 ($P < 0.05$)	Significant
C	C2M	4	8	179.55 ± 7.94 ($P > 0.05$)	Not significant
	C3M	4	8	166.66 ± 7.87 ($P > 0.05$)	Not significant

* = S.E. Mean standard error.

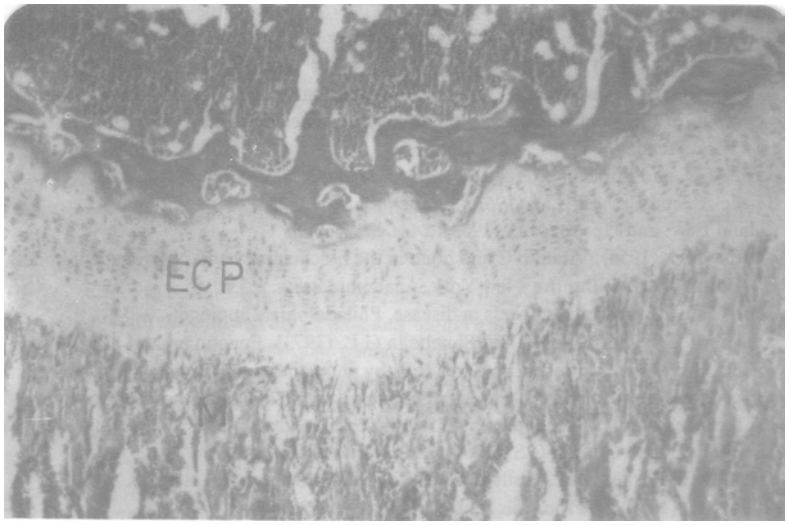

Fig. 1: Proximal epihyseal plate (EPC) of normal untreated male mouse S weeks old, showing well developed bone trabeculae in the epiphysis (E) and in metaphyseal area (M) H x E stain, photomicrograph x 87.5.

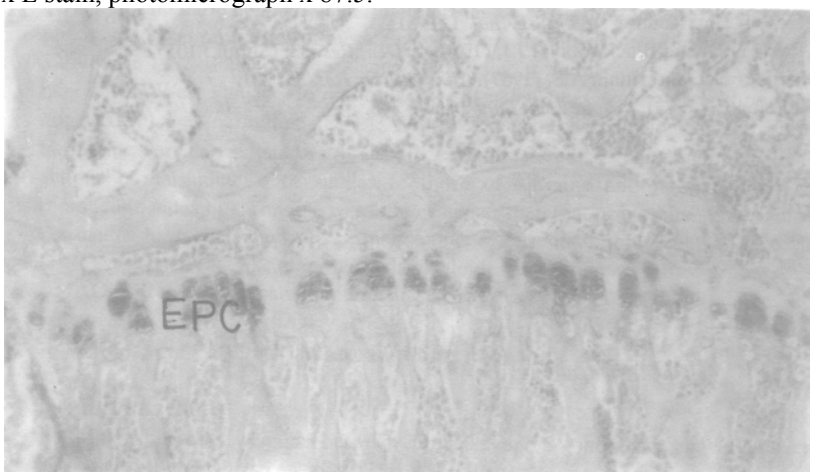

Fig. 2 Proximal end of the tibia from Dexa methasone treated mouse after 45 injections, showing narrow width of the epiphyscal cartilage (EPC) and concomittend reduction in the size of the cells making up zones.
H & E stain, photomicrograph x 87.5

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