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STUDIES OF THE EFFECTS OF DEXAMETHASONE SODIUM PHOSPHATE ON WEIGHT OF BONE (TIBIA) IN MICE

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ABSTRACT

Seventy two immature male and female mice were taken and divided into three groups, 24 mice remained untreated and 24 male and female mice were treated upto six weeks with daily dose of Dexamethasone phosphate. Twenty four similar mice were given injections of vehicle in which the drug, dexamethasone was suspended and this acted as placebo group.

Dexamethasone treated mice group showed less tibia weight as compared to control and placebo group.

Introduction

The bone disorder, Osteoporosis signifies in general loss of skeletal tissue in which trabecular bone is more affected than cortical bone, there arc different types of osteoporoses, primary osteoporosis where the cause is not fully known and secondary osteoporosis where an acknowledged predisposing factor or disease is present. The secondary osteoporosis is the feature of some endocrine disease.

The syndrome known as Cushing's syndrome has the tendency of spontaneous fractures of the bones.

The use of corticosteroid in therapy has produced an iatrogenic form of osteoporosis first described by Boland and Headley (1950) and subsequently by many others, its incidence is impossible to establish because it must depend on the type of steroid, the duration of therapy, the dosage and many other factors but Hoiden (1960) found osteoporosis reported in 12 out of 237 patients on steroid therapy. In Cushing's Syndrome, osteoporosis is said to occur in atleast 40% of cases as mentioned by Sprague et al. (1956).

Cortisol when administered in higher concentrations and for a prolonged period of time develops certain pharmacological properties in addition to its physiological ones (Goodman and Gilman 1968). Among the more prominent of the effects upon skeletal tissues arc (i) a marked decrease in glucose utilization (Kornel 1973), (ii) an increased breakdown of proteins and lipids concomitant with a decreased synthesis of R.N.A., proteins and lipids (Baxter and Forsham 1972). (ii) Reduction in the synthesis of collegen and sulphated mucopolysaccharides (Dougherty et al. and Schnebella et al. 1973).

Administration of corticosteroids in experimental animals produced wide spread osteoporosis particularly of trabecular bone associated with increased osteoclastic resorption. There is also evidence that those compounds tend to lower plasma calcium possibly by reducing absorption and that they inhibit the action of parathyroid hormone.

Administration of corticosteroids in very-large doses raise urine calcium almost certainly by inhibition of tubular reabsorption in addition cortisol tend to decrease calcium absorption in the intestine (Storey 1968).

The effect of corticosteroids on skeletal growth and bone becomes of interest as a result of constant use of hormones as therapeutic anti-inflammatory agents. The few investigations which have stressed the influence of the corticosteroids upon young growing bone (Wells and Kendall 1940), (i) by using young rodents demonstrated that cortisone inhibited linear growth by interfering with proliferation of the epiphysel cartilage and with the production of cartilagenous matrix (Follis 1951). (ii) Found that primary spicules were poorly ossified and were inadequately resorbed. (iii) Concurred in saying that these spicules may persist for an abnormally long period of time on the other hand little attention has been directed towards the fundamental process whereby these hormones not only influence the transformation of young bone into adult state but also exert an aging upon mature bones.

Effect of Corticosteroids on Bones:

It is recognized that prolonged glucocorticoid administration leads to reduce skeletal growth in children which present a serious handicap in treatment of childhood allergy. The medical literature contains many reports of injurious side effects of systemic corticosteroid therapy (Heiman and Ember-get 1960).

Excretion of bone forming minerals may also occur with use of corticosteroids (Gordan 1961).

Anatomy of the Tibia of Mice:

The skeleton of the leg of the mice is formed by stout tibia along with another bones and extremely slender bone fibula. Proximally tibia possess two condyles articulating with femoral condyles. On the anterior surface of the tibia is a narrow ridge or crest. Distally the tibia expands and forms median projection the medial malleolus. The thin fibula is lateral to the tibia fuses with it distally and then forms as expended lateral malleolus.

Materials and Methods

Seventy two immature mice (36 females and 36 males) of J.P.M.C. breed weighing between 15-17 Gm and aged between 4 and 4.5 weeks were taken from the animal house of Jinnah Post Graduate Medical Centre. The mice 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 3 subgroups.

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 subgroups.

Sub group A 2F:

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

Sub group A 3F:

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

Sub group Al M:

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

groups.

Sub group A2 M:
This group consisted of 4 female mice. These animals were killed 4 weeks after the commencement of the experiment

Sub group A3 M:
This group consisted of 4 mice and were killed 6 weeks after the commencement of the experiment.

GROUP B (TREATED)
This group consisted of 24 mice (12 female and 12 male). The animals were given dexamethasone sodium phosphate by daily intramuscular injection. The dose was calculated at 0.16 mG/G body weight of the hormone in a volume of 0.12 ml. These animals were further divided into three subgroups similar to those of control.

Sub group B1 F:
Four female mice were sacrificed after two weeks of experiment.

Sub group B2 F:
Four female mice were sacrificed after four weeks of experiment.

Sub group B3 F:
Four female mice were sacrificed after six weeks of experiment.

Sub group B1 M:
Four male mice were sacrificed after two weeks of experiment.

Sub group B2 M:
Four male mice were sacrificed after four weeks of experiment.

Sub group B3 M:
Four male mice were sacrificed after six weeks of experiment.

GROUP C (Placebo)
This group of mice consisted of 24 mice (12 female and 12 male) and was given placebo treatment by Um injection of 0.32 ml of the solvent used for the corticosteroids.

Like the other groups this group was further divided into three sub groups.

Sub group Cl F:
Four female mice were sacrificed after two weeks of experiment.

Sub group C2 F:
Four female mice were sacrificed after four weeks of experiment.

Sub group C3 F:
Four female mice were sacrificed after six weeks of experiment.

Sub group Cl M:
Four male mice were sacrificed after two weeks of experiment.

Sub group C2 M:
Four male mice were sacrificed after four weeks of experiment.

Sub group C3 M:
Four male mice were sacrificed after six weeks of experiment.

Administration of Drug:
Injection dexamethasone sodium phosphate MSD (Decadrone) was used each ml containing 4 mg of the drug in 1ml.

For mouse 0.16 ug/G of body weight was administered i.e. about twice of human corresponding dose. Amount of drug for 15 Gm of mouse was = 15 x 0.16 = 2.4 ug.

Placebo contained:

Creatinine	8.0 mg
Sodium citrate	10 mg
Sodium hydroxide	10 mg
Methyl paraben	15 mg
Propyl paraben	0.2 mg

dissolved in 1 ml of solution.
0.1 ml of placebo solution was used.

Removal of Tibia:
The tibias were removed and weighed by torsion balance and was processed.

Results and Discussion

The mean weight of group AlF tibia was 5487 ± 2.26 mg, it was compared with B1F

and it was 59.62 ± 1.68 mg ($P > 0.05$). It was not statistically significant (Table 1).

Similarly the mean weight of group AIM was 54.12 ± 2.22 mg, it was compared with group B1M this 65.57 ± 3.16 mg, this was statistically significant ($P > 0.05$ table 2).

The mean weight of A1F was compared with C1F, the mean weight of C1F was 61.5 ± 1.36 mg. It was statistically significant ($P < 0.05$ table 1).

The mean weight of group AIM was compared with CIM, where the mean weight of this group was 66.0 ± 1.87 mg, it was statistically significant ($P < 0.05$ table 2).

The mean weight of group A2F was 60.87 ± 1.62 mg, and it was compared with B2F the mean weight was 53.87 ± 1.31 mg. It was statistically significant ($P < 0.05$ table 2).

The mean weight of group A2M was 64.50 ± 2.53 mg where the mean weight of B2M was 44.25 ± 2.30 mg. It was statistically significant ($P < 0.05$ table 2).

The mean weight of group A2F was compared with C2F, the mean weight of C2F, was 61.87 ± 1.98 mg. It was not statistically significant ($P > 0.05$ table 1).

The mean weight of group A2M was compared with C2M, the mean weight of C2M was 57.12 ± 1.07 mg and it was statistically significant ($P < 0.05$ table 2).

The mean weight of A3F was 55.75 ± 2.65 whereas the mean weight of B3F was 54.0 ± 1.64 mg, it was statistically not significant ($P < 0.5$ table 1).

The mean weight of A3M was 61.62 ± 2.31, it was compared with B3M, it was 56.62 ± 1.79 mg. It was statistically not significant ($P > 0.05$ table 2).

The mean weight of A3F was compared with C3F, the mean weight of this group was 63.0 ± 1.24 mg, it was statistically significant ($P < 0.05$ table 1).

The mean weight of group A3M was then compared with C3M which was 67.0 ± 1.72 mg. It was statistically not significant ($P > 0.05$ table 2).

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 greater availability of amino acids which are converted into carbohydrates. The administration of steroids (Dexamethasone) over a prolonged period will also reduce protein anabolism of the body. So that osteoporosis will be caused. This commonly results decrease of bone weight.

Conclusion

After treatment with Dexamethasone the weight of the bones is reduced as has been shown by this study that weight of tibia has been reduced.

Table 1
Weight of the tibia female mice

Group No.	Sub group	No. of animals	No. of tibia right and left	Mean weight of tibia (mg)	Statistical significance
A	A1F	4	8	54.87 ± 2.26	
	A2f	4	8	60.87 ± 1.62	
	A3F	4	8	55.75 ± 2.65	
B	B1F	4	8	59.62 ± 1.68	Not significant ($P > 0.05$)
	B2F	4	8	53.87 ± 1.31	Significant ($P < 0.05$)
	B3F	4	8	54.0 ± 1.64	Not significant ($P > 0.05$)
C	C1F	4	8	61.5 ± 1.36	Significant ($P < 0.05$)
	C2F	4	8	61.87 ± 1.93	Not significant ($P > 0.05$)
	C3F	4	8	63.00 ± 1.24	Significant ($P < 0.05$)

Table 2
Weight of the tibia in male mice

Group No.	Sub group	No. of animals	No. of tibia right and left	*Mean weight of tibia	P value of difference as compared to control group (A)
	A1M	4	8	54.12 ± 2.22	
	A2M	4	8	64.50 ± 2.53	
	A3M	4	8	61.62 ± 2.31	
	B1M	4	8	65.37 ± 3.16	Significant ($P < 0.05$)
	B2M	4	8	44.25 ± 2.30	Significant ($P < 0.05$)
	B3M	4	8	56.62 ± 1.79	Not significant ($P > 0.05$)
	C1M	4	8	66.00 ± 1.87	Significant ($P < 0.05$)
	C2M	4	8	57.12 ± 1.07	Significant ($P < 0.05$)
	C3M	4	8	67.00 ± 1.72	Not significant ($P > 0.05$)

*S.E. standard error

References

Baxter J.D. and Forham F.H. (1972). *Am. J. Med.,* **53**: 573.

Boland E.W. and Headley N.E. (1950). *JAMA*. **144**: 365.

Dougherty T.F., Starvens W. and Schnebclla G.L. (1975). Functional and morphological alterations produced in target cells by anti-inflammatory steroids: in recent progress in hormones research proceedings of 1972. Laurentian hormones, Conference edited by Academic Press. New York, London. p.287.

Follis R.H. (1950. *Jr. Proc. Soc. Exp. Biol. Med*., **76**: 722.

Goodman L.S. and Gilman A. (1968). The pharmacological basis of therapeutics. 5th ed. New York, Macmillan. p.1459.
Gordan G.S. (1961). *J. Med.,* **57**: 740.
Heiman W.G. and Freeberger R.H. (1960). *N. Eng. J. Med,* **263**: 672.
Kornel L. (1973). *Acta Endocrinol.,* **74** suppl. 178.
Norden B.E.C. (1960). *Br. J. Dermatol.,* **72**: 40.
Sprague R.G., Randall R., Vsalan R.M., Scholz D.A. Priestley Walters W. and Bulbulian A.H. (1956). *Arch. Intern. Med.,* **98**: 369.
Stoery E. (1968). *Clin. Orthop. Res.,* **30**: 197.
Wells B.B. and Kendall E.C. (1940). *Proc. Mayo Clin.,* **15**: 324.