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Histomorphology of Bone and Use of Corticosteriods: Does Dose and Duration Matter? A Prospective Study on Wistar Rats

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Abstract: ***Background:*** ***Steroid Osteoporosis has been an area of concern over the years. Despite their great clinical usefulness, unwanted effects are likely to occur especially with large doses and prolonged administration. They cause a reduction in the mass of the bone by reducing the number of bone forming cells and by causing alterations in calcium absorption and renal handling of calcium. . 20% of all cases of osteoporosis have been attributed to glucocorticoid exposure.*** ***Materials and Methods:*** ***The materials used in this study include; the subjects (Wistar rats), the drug (hydrocortisone), decalcifying fluid, alcohol, pure xylene, paraffin wax, haematoxylin and eosin stain. This study was carried out on sixteen Wistar rats, four of which were used as controls while twelve were used for the experiment. Hydrocortisone was administered intramuscularly in therapeutic and double therapeutic doses to the experimental models and sections of bone taken for study.*** ***Results:*** ***The sections of bones taken showed a reduction in the size of the harvesian system and varying degree of proliferation of the osteoclasts according to duration and dose.*** ***Conclusion:*** ***Although corticosteroids are therapeutically effective, their use is associated with certain complications on different organs and systems of the body. It was observed in this study that they cause a reduction of the harvesian systems of bone and proliferation of the osteoclasts. Also, these effects are increased by large doses and prolonged administration with osteoporotic potentials. Future work on this will be on concomitant use of osteoporotic medications and their safety profile.***

Keywords: Histomorphological effects, Histology of bone, Steroid osteoporosis, Bone, Corticosteroids.

1. Introduction

Glucocorticoids are one of the functional categories of corticosteroids in addition to Mineralocorticoids and Sex steroids. Glucocorticoids regulate the metabolism of carbohydrates, proteins and other molecules, it is secreted by the zona fasiculata while Mineralocorticoids regulate water, sodium and potassium balance, and it is secreted by the zona glomerulosa, In addition to their metabolic effects, corticosteroids have anti-inflammatory and immune-suppressive activities and it is for these reasons that they are most commonly used therapeutically. 20% of all cases of osteoporosis have been attributed to glucocorticoid exposure [1].

Steroid Osteoporosis has been an area of concern over the years. It is on record that from the time of Harvey Cushing's first description of the syndrome which now bears his name, an association of glucocorticoid excess and osteopenia had been suggested.[2] Subsequently, Fuller Albright [3] postulated that the anti-anabolic action of glucocorticords contributed to the development of osteoporosis, and when the hormones were introduced and used therapeutically in the 1940s and 1950s for diverse group of conditions including asthma, rheumatoid arthritis and many other inflammatory diseases with wonderful outcomes, case reports of clinical osteoporosis soon followed. [4] Since then, numerous investigations have shown that bone mass is subnormal in steroid treated patients [5],[6],[7] Despite their great clinical usefulness, unwanted effects are likely to occur especially with large doses and prolonged administration. They cause a reduction in the mass of the bone by reducing the number of bone forming cells and by causing alterations in calcium absorption and renal handling of calcium. [8]

2. Objective

The study is aimed at demonstrating the short term and medium term effects of therapeutic and double therapeutic doses of corticosteroids on the histology of the bone.

3. Hypothesis

Based on the fact that corticosteroids increase the risk of osteosporosis by increasing the osteoclastic activities and decreasing the osteoblastic activities, we expect that there will be a reduction in the total number of the harvesian system and lamella. The number of incomplete harvesian systems present will be more than the complete systems. Alternatively, there could be an increase in the number of harvesian systems and lamella.

4. Scope of Study

The study is a prospective study involving Sixteen (16) Wistar rats; Twelve (12) as experimental models and four (4) as control. The effect of corticosteroids on the harvesian system and lamella of the cortical bone of the models will be studied on microscope.

5. Materials and Methods

The materials used in this study include; the subjects (Wistar rats), the drug (hydrocortisone), Decalcifying fluid, Alcohol,

Pure Xylene, Paraffin wax, Haematoxylin and Eosin stains. This study was carried out on sixteen (16) Wistar rats, four (4) of which were used as controls while twelve (12) were used for the experiment.

The weights of the experimental models were; 175 kg, two weighed 190 kg, five weighed 200 kg, three weighed 210 kg and 225 kg respectively, while the controls weighed 200 kg.

They were divided into two groups A and B. Those in group A were given normal dose of Hydrocortisone (2mg/kg) while those in group B were given double the normal dose (4mg/kg). Those in group A were divided into subgroups A4 and A6 which were given the drugs for four (4) and six (6) weeks respectively. Those in group B were also divided into subgroups B4 and B6 which were given double dose for four (4) and six (6) weeks respectively.

Hydrocortisone was obtained in powdered form of 100mg and was dissolved in 100ml of distilled water which gave a concentration of 1mg/kg. It was then administered intramuscularly once daily accordingly. Ebner's decalcifying fluid was used in the process of decalcification for 3 days after which graded concentrations (50%, 70%, 95% and 100%) of alcohol (ethanol) were used for the process of dehydration for 2 hours each but for the 95% 12 hours and 4 hours respectively.

- Pure xylene was used as a cleaning agent and molten paraffin was used for the process of impregnation.
- The stains that were used are Haematoxylin and Eosin.
- The rats were sacrificed at the end of the 6weeks and their femurs harvested and preserved.
- Finally, the sections on the slides were subjected to photomicrography. The resultant micrographs were then analyzed using the following indices; number of harvesian system and number of lamella present.

6. Results

The results obtained are as follows;

a) For the control, the section of the bone showed several harvesian system, an irregular interstitial tissue and a thin periosteum.
b) For A4 which was given normal dose for 4 weeks, the section of bone showed a reduced harvesian system, an expanded interstitial tissue and mild proliferation of osteoclasts.
c) For A6 which was given the normal double dose for 6 weeks, the section of bone showed thinning and reduction in the size of the harvesian system. There was expansion of the interstitial tissue and moderate proliferation of the osteoclasts.
d) For B4 which was given double dose for 4 weeks, the section of bone showed thinning of the harvesian system and moderate proliferation of the osteoclasts.
e) For B6 which was given double dose for 6 weeks, the section of bone showed thinning and reduction of the harvesian system and marked proliferation of the osteoclast,

Figure 1: Histologic section of bone showing several harvesian systems, an irregular interstitial tissue and a thin periosteum.

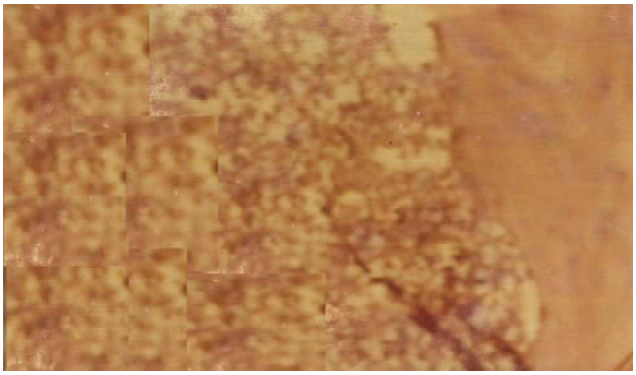

Figure 2: Histologic section of bone showing a reduced harvesian system, an expanded interstitial tissue and mild proliferation of osteoclasts

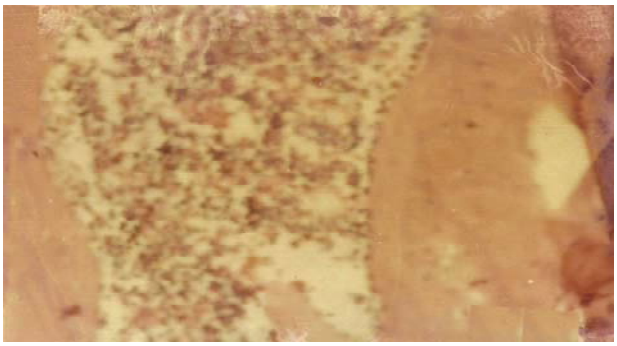

Figure 3: Histologic section of bone showing thinning and reduction in the size of the harvesian system, expansion of the interstitial tissue and moderate proliferation of the osteoclasts.

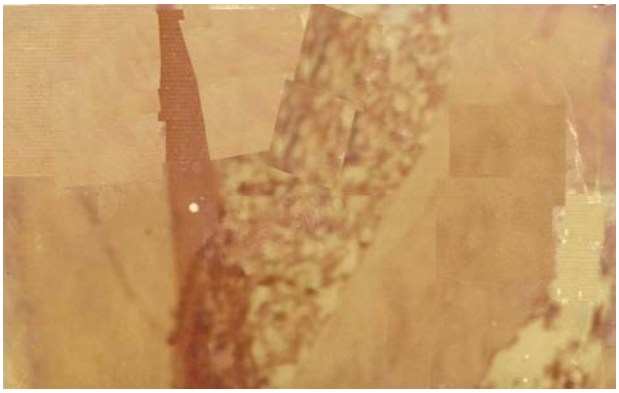

Figure 4: Histologic section of bone showing thinning of the harvesian system and moderate proliferation of the osteoclasts.

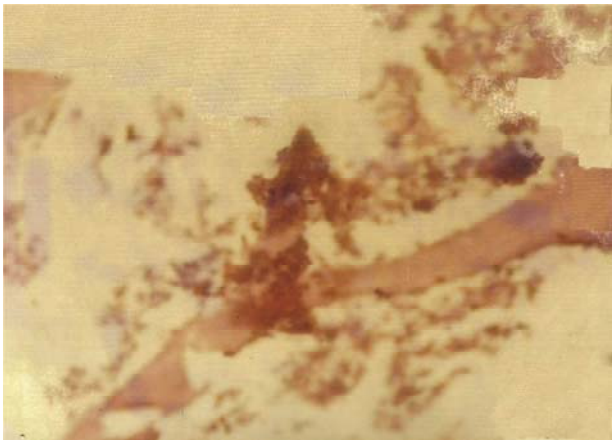

Figure 5: Histologic section of bone showed thinning and reduction of the harvesian system and marked proliferation of the osteoclasts.

7. Discussion

In this study, it was found that the histologic section of the bone (figure 1) showed several harvesian systems, an irregular interstitial tissue and a thin periosteum. These findings in the controls are in contrast to the findings in the experimental subjects and this can be explained by the fact that there was no hydrocortisone exposure in this group. On the other hand, histologic sections of bone from the experimental models showed varying degree of changes with increasing severity according to duration and dose of hydrocortisone (figure 2-5).

For the group A that received therapeutic dose of hydrocortisone, it was found that the histomorphologic effects were more in the subjects that received the drug for 6 weeks when compared to 4 weeks (figure 2&3).

For the group B that received double therapeutic dose of hydrocortisone, it was also found that the histomorphologic effects were more in the subjects that received the drug for 6 weeks when compared to 4 weeks (figure 4&5).

When group A and B were compared, it was found that the histomorphologic effects were more in the subjects that received double therapeutic doses of hydrocortisone (group B) for 4 and 6 weeks when compared to normal therapeutic doses of hydrocortisone (group A) for 4 and 6 weeks duration respectively.

Further close observation of the results revealed that there were similar histologic changes of thinning and reduction of the harvesian system and moderate proliferation of the osteoclasts seen in subjects that received normal therapeutic dose of hydrocortisone for 6 weeks (A6) and subjects that received double therapeutic dose for 4 weeks (B4).

These findings are in support of the fact that corticosteroids are potential osteoporotic agents as hypothesized in this study. The thinning and reduction in the size of the harvesian systems could be attributed to the increased activity of osteoclasts caused by glucocorticoids. Increase in osteoclastic activities with concurrent decrease in osteoblastic activities may lead to an increase in the rate of bone resorption. Increase in the rate of bone resorption will subsequently lead to a reduction in the bone mass which is the pathological basis of osteoporosis. [9],[10],[11],[12],[13]

Similar findings were obtained in other reports. It is on record that corticosteroids have a number of effects on calcium metabolism and bone cell formation. The relative contribution of each of these to the development of osteopenia is unknown and it's elucidation is complicated by the existence of significant interspecies differences in the response to corticosteroids [14] Feldman et al [15] have demonstrated the presence of glucocorticoid receptors in bone, making a direct action of steroid hormones on bone a possibility. Protein and collagen synthesis, cell growth, and RNA synthesis are all inhibited by glucocorticoids. [16], [17] Parveen and Micheal [8] in their study noticed that corticosteroid was shown to affect the harvesian system and the lamella by reducing the bone forming cells. This is in keeping with our findings. Prolonged corticosteroid administration is a major predisposing factor for spontaneous femoral head necrosis/localized osteonecrosis. [18], [19] Again, this supports our findings.

Perhaps, it will be out place to stop using corticosteroids because of their mind blowing therapeutic efficacy especially as among the first line of drug in allergic conditions like asthma. Therefore, we recommend prophylactic therapy with calcium and vitamin D for patients on chronic use of therapeutic corticosteroids to avert corticosteroids induced osteoporosis.

8. Conclusion

Although corticosteroids are therapeutically effective, their use is associated with certain complications on different organs and systems of the body. It was observed in this study that they cause a reduction of the harvesian systems of bone and proliferation of osteoclasts. Also, these effects are increased by large doses and prolonged administration with osteoporotic potential. Future work on this will be on concomitant use of osteoporotic medications and their safety profile.

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Author Profile

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