Effect of Hydrocortisone Succinate on Ca$^{45}$ Resorption and Incorporation in Bone Culture of Rat

Moo Youn Cho, Chang Do Choi and Je Hyun Kim

Department of Biochemistry, Yonsei University College of Medicine, Seoul, Korea

ABSTRACT

Ca$^{45}$ resorption and incorporation into albino rat-bones in tissue culture was considered in studying the pathogenesis of osteoporosis caused by continuous administration of glucocorticoid, hydrocortisone succinate. 18-day old tibias were cultured in a chemically defined media, (BGJb). Hydrocortisone showed no effect on Ca$^{45}$ resorption and little increase of Ca$^{45}$ incorporation into bone. This may suggest that hydrocortisone produces osteoporosis not by direct effect but by secondary effects on calcium metabolism.

INTRODUCTION

Although osteoporosis by glucocorticoids administration was obtained in clinical and animal experimentations (Fraser, 1962 Heaney, 1965 Kelly et al, 1967 and Hardt, 1972 Chalmers and Ho, 1972) the pathogenesis is still uncertain as to whether this effect is due directly on the bone (Matrix or minerals) or indirect interferences of bone metabolism.

Effects on bone metabolism by glucocorticoids have been investigated many times. Calcium depletion, (Van Bucham, 1959 Storey, 1960 and Collins et al, 1962) decreased the Ca$^{45}$ transfer across the intestinal wall, (Harrison and Harrison, 1960 Kimberg et al, 1961) increased renal clearance of calcium, (Yrinis, 1964) decreased growth of cartilaginous limb bone rudiments, (Fell and Thomas, 1961) increased breakdown of bone matrix and bone resorption. Bone resorption has been reported by many workers. Bone resorption stimulated by vitamin A is more susceptible to glucocorticoid inhibition than resorption stimulated by parathyroid extract. (Raisz, 1965) Cortisol inhibits the stimulation of boneresorption produced by vitamin A, prostaglandin E, and dibuty1 cyclic 3'-5' adenosine monophosphate in culture. (Raisz et al, 1972) However there are still controversial results; bone resorption may be enhanced (Jee et al, 1970) or inhibited. (Raisz, 1965, Stern, 1969) The present study is to clarify the effects of glucocorticoid on bone ca-
Effect of Hydrocortisone on Ca\textsuperscript{45} in Bone Culture

METHODS

Fetuses of albino rats were used throughout the study. The O-day of pregnancy was determined by the presence of sperm in the vaginal smear. The shafts of the tibia were dissected from 18-day old fetal rats taken from the mothers. In Ca\textsuperscript{45} release experiments, Ca\textsuperscript{45}(50μCi)(specific activity, 161μCi/μg) was injected subcutaneously into the mothers 18 to 20 hours prior to sacrifice.

The bones were cultured for 4 or 6 days at 37°C in a chemically defined media, BGG (Bigger et al., 1961) supplemented with 50% human serum (Stern and Raisz, 1967) in a chamber of 5% CO\textsubscript{2} in air. One ml of culture media was transferred into a culture dish or slide, and the dissected paired tibia put on a millipore-filter paper (0.5cm x 1.0 cm) which was moistened in media and cultured. In Ca\textsuperscript{45} incorporation experiments Ca\textsuperscript{45} (0.02μCi/25μl) was added along with hydrocortisone (0.5mg/25μl) to the culture media. Control studies used 0.85% physiological saline (25μl) in place of hydrocortisone.

The growth of the tibias was measured with a metric map measurer (0.01mm scaled) through the cover of the dishes. We compared the measurement of tibia length through the cover or without the cover, and there was no difference significantly between them.

For measuring Ca\textsuperscript{45}, bone was rinsed several times with the media solution and rolled on filter paper and weighed, then ashified in a muffle furnace at 500-700°C over night (12-15 hours). Hydrocortisone succinate was purchased from Upjohn company and it was dissolved in the buffer and diluted in a saline solution. The medium was collected with a Pasteur pipette and ashified with the same treatment as the bone ashes and measured for Ca\textsuperscript{45} activity. Radioactivity was determined by Packard-Tricab liquid scintillation spectrometer. The scintillation cocktail was composed of 5g of 2,5-diphenyloxazol(ppo) and
Table 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ca⁴⁺ Incorporation</th>
<th>Number of fetus</th>
<th>Mean activity in Bone (cpm)</th>
<th>Boiled/Control ratio</th>
<th>HC⁻⁻/Control ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (NaCl)</td>
<td></td>
<td>8</td>
<td>1740±⁺²¹⁷.⁶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiled</td>
<td></td>
<td>5</td>
<td>996±⁺¹⁰².⁰</td>
<td>0.70±⁺⁰.⁰₆⁷</td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone treated</td>
<td></td>
<td>8</td>
<td>1917±⁺⁵⁹¹.⁴</td>
<td>1.11±⁺⁰.⁰₃¹</td>
<td></td>
</tr>
</tbody>
</table>

* Standard error of each group
HC: hydrocortisone treated

0.3g of p-bis [2-(4-methyl-5-phenyloxazole)] -benzen per liter of toluene.

RESULTS

The tibia of the 18-day old fetus was of an average length of 1.98±₀.₀₅mm and the most rapid growth was in the first two days. The growth rate per 2 days in subsequent days was 0.5mm. This growth rate was similar to Biggers data (1961). Data are shown in Fig.1. Ca⁴⁺ release was neither significantly decreased nor increased in the hydrocortisone treated group compared to the control in both bone and media as shown in Table 1. Ca⁴⁺ was released almost 90% of the total amount after the first 2-day-culture. Hydrocortisone/ control ratio was a mean value of 1.08 in the bone and 0.98 in the media. This showed there was no resorption phenomena. In Ca⁴⁺ incorporation experiments Ca⁴⁺ activity ratio was 0.7 in boiled bone/control bone and 1.11 in hydrocortisone treated/control. This indicated that hydrocortisone may enhance Ca⁴⁺ incorporation into bone or may interfere with the resorption if there were no difference in Ca⁴⁺ incorporation. This data is in Table 2.

DISCUSSION

Several investigations have demonstrated that glucocorticoids inhibit bone resorption in tissue culture (Stern, 1969; Raisz, 1969) but the results have not been consistent. Others have reported that the glucocorticoids enhance bone resorption. (Jee et al, 1970) The present study indicates that 10⁻⁴M hydrocortisone succinate showed no significant bone resorption in tissue culture by testing the release of Ca⁴⁺ or the incorporation of Ca⁴⁺ into bone minerals. Cortisol was shown to inhibit the uptake and the incorporation of both RNA and protein precursors in isolated bone cells grown to confluence in monolayer cell cultures. Little inhibition of amino acid or uridine incorporation was observed in fetal long bone shafts in organ cultures at 10⁻⁴M concentration of cortisol. (Peck et al, 1969) Raisz et al (1972) suggested that cortisol may be a specific inhibitor of transcriptional response in single cells without affecting the overall RNA and protein synthesis or it might oppose the induction and escape by some other mechanism. Choi et al (1973) reported that hydrocortisone may not stimulate protein degradation of C¹⁴-labelled protein in bone culture.

Based upon the present results the pathogenesis of osteoporosis caused by chronic administration of glucocorticoids may be explained as follows; first the changes of bone matrix resulting from decreased mucopoly-
Effect of Hydrocortisone on Ca\textsuperscript{44} in Bone Culture


Storey, E.: Bone changes associated with cortisone...
