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BONE TISSUE OF THE INTERRADIX REGION IN ANIMALS TREATED WITH HYDROCORTISONE

SIMIĆ RUŽICA*, ŠIMIĆ MARIJA**, KARADŽOV OLGA* and JOKIĆ NEVENKA*

* *Dental Diseases Clinic, Faculty of Stomatology, University of Belgrade ** Department of Histology and Embriology, Faculty of Veterinary Medicine, University of Belgrade*

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The aim of this investigation was to examine the effects of long term application of different doses of hydrocortisone (Hy), (2, 4, 6, 8, 16, 32, 50 mg Hy kg/b. wt.) on bone cells and matrix in the interradix molar jaw region of rats treated in the period of intensive growth.

Stereologic analysis was applied using methods for determining the numeric (Nv) and volume (Vv) density of connective cells (osteoblasts, osteocytes and osteoclasts) and the volume density of the bone tissue matrix.

The results of quantitative analysis showed that only the supraphysiological doses of 16 and 32 mg Hy/kg b. wt. caused highly significant decreases of the numeric density for all three categories of cell elements of bone tissue compared with controls. Osteocytes as the most numerous cells, were analysed through volume density parameters. Because the number and volume of osteocytes in the groups treated with Hy were diminished in comparison to controls, and the biggest differences were in the groups treated with 16 and 32 mg Hy/kg b. wt., it was concluded that large doses of Hy may cause reduction of the population of small osteocytes. This corresponds with "slightly different percentage ratio of bone matrix volume density", also noticed in the examined region of the jaw bone.

Key words: Hydrocortisone, bone cells, rat.

INTRODUCTION

Therapy of many diseases consists of very widespread and often unjustified use of glucocorticoids (Reginster et al. 1989.). Positive pharmacological effects may be noticed depending on the dose and duration of therapy, but there is also the possibility of negative side effects provoked by suppression of the hypothalamo-hypophyseal adrenal axis, presented with an unwanted corticoid influence on the metabolism of some cells and tissues in the organism, particularly in animals that are still growing (Spector, 1981.).

Pharmacological doses of glucocorticoids have an impressive influence on the cellular metabolism on bone tissue (Pack, 1967., Chen & Feldman, 1979., Wong, 1979.), and on the number and size of bone cells (Suda et al., 1983.). The extent of the effect depends on the dose and duration of exposure to hormone influence (Chen et al., 1983.).

The experimental results from our previous research on the influence of hydrocortisone on growth and development of rat jaws indicate that changes in growth are connected with the size of dose used. Low doses of 2 mg Hy/kg did not have any significant influence on rat jaw growth, while increased amounts of Hy (4, 8, 16 and 32 mg/kg) decreased the value of the examined jaw parameters almost as a rule. In the same experiments it was shown that hydrocortisone affects dental tissues depending on dosage, that is, clear histological changes occur in the structure of the pulpo-dental complex and interdental gingiva tissue (Jokić, 1987., Karadžov et al., 1985., 1987., 1989.).

The direct aim of this study was to examine the effects of long term therapy with different doses of hydrocortisone on bone cells and matrix in the interradix region of the jaw molar in rats treated during the period of intensive growth.

MATERIALS AND METHODS

For the experiments 30 white Wistar rats 15 days of age, were divided into groups according to the dose of hormone used. Five days a week animals were treated with hydrocortisone acetate (Galenika, Beograd) in subcutaneous daily dosages of 2, 4, 8, 16, 32 and 50 mg Hy/kg of body weight. The treated group and control group were sacrificed at the age of 45 days. Molar regions of the mandible were fixed in Bouin, decalcified with EDTA and stained with hematoxylin -eosin (HE).

Stereological analysis of bone cells and matrix on meziodistal sections of the interradix region of the molar was performed using Weibel's multipurpose test system (P:42) and an ocular micrometer 5:100.

Volume density (Vv) was determined because this stereological method can define which part of the total space is occupied by the examined phase in unity of volume.

The stereological method of numeric density (Nv) determination represents the number of parts in the total area. It was used to determine the number of cells per mm^3. Total area (At) was calibrated by an objective micrometer. With the lens providing 50 x magnification it was 0,014548 nm. Student's t-test was used for defining the significance of differences between the results obtained for control and treated groups.

The numeric density in young bone of osteoblasts, mature osteocytes and osteoclasts, was determined in the control group and the groups treated with different doses of hydrocortisone. The volume density of osteocytes and matrix of bone tissue were also measured, that is their proportional diffusion in the unity of bone tissue volume obtained.

RESULTS

Analysis of bone element number. In the interradix region of the molar teeth in nontreated animals the value of numeric density, for osteoblasts was 2,30 x 10^4, for osteocytes 6,40 x 10^4 while for osteoclasts it was the lowest, 1, 11 x 10^4 per mm^3, (Figure 1.).

The average values of numeric density (Nv) for the investigated bone cells in the interradix region of the molar teeth of control and treated animals are summarized in Figure 1. The Nv in control animals was 2.30 x 10^4 /mm^3 for osteoblasts, 6,40 x 10^4 /mm^3 for osteocytes and 1.11 x 10^4 /mm^3 for osteoclasts.

The average number of osteoblasts per mm^3in the interradix region of molars in treated animals was decreased in all experimental groups in a dose dependant manner, except for the group treated with 50 mg Hy. The differences between the control group and the groups treated with 2 and 4 mg Hy were not statistically significant. The negligible decrease of osteoblast number in some groups indicates that the low dose of Hy did show an influence on osteoblasts in the interradix region of the molar.

A statistically significant difference ($p < 0,01$) was found between the average values for osteoblast number in the group treated with 8 mg Hy and the control group.

The difference was statistically highly significant ($p<0,001$) for the groups treated with 16 and 32 mg Hy in relation to the control group. Individual values for numeric volume of osteoblasts in those experimental groups were 1,62 x 10^4, and 1,73 x 10^4 per mm^3 respectively.

The average number of osteocytes (Nv), in the interradix region was also decreased in relation to corresponding control groups. However it seems that osteocytes are more sensitive to the hormone because even low doses of 2 and 4 mg Hy caused statistically significant decreases in comparison with the control group ($p<0,001$). (Figure 1.)

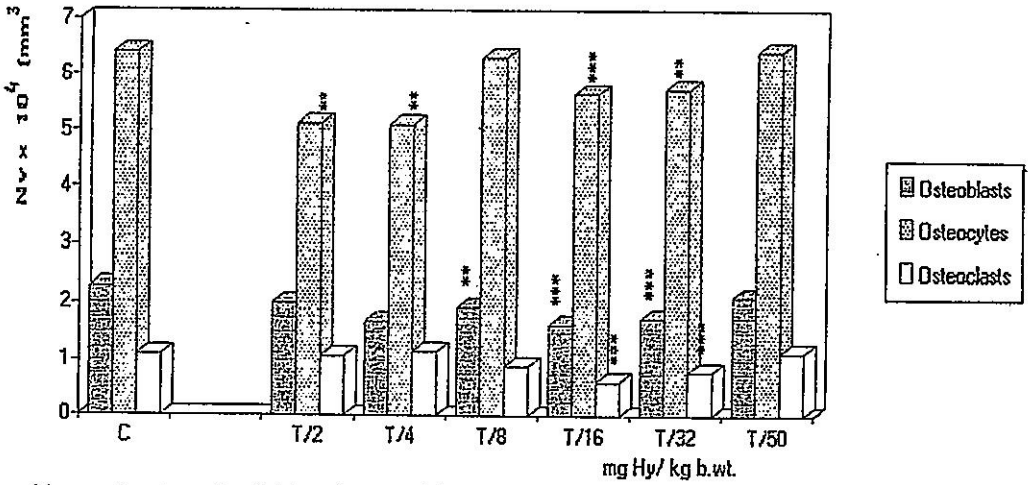

Figure 1. Numeric density (Nv) of osteoblasts (ob), osteocytes (oc) and osteoclasts (ok) in mm 3 in control animals (C) and animals treated (T) with 2, 4, 8, 16, 32 and 50 mg Hy/kg of body weight.

Osteoclasts, as the third category of cells in developing bone, were also affected by hydrocortisone. The doses of 16 and 32 mg Hy caused a statistically significant decrease in the number of osteoclasts ($p < 0,001$) in comparison to the corresponding control group. The numeric density in the group treated with 16 mg Hy was $0,6 \times 10^4$ and in the group treated with 32 mg Hy was 0,77 x 10=4. Osteoclast numeric density did not change in the groups treated with low doses of 2 and 4 mg Hy and with the supraphysiological dose of 50 mg Hy.

Analysis of the bone element volume. The distribution of osteocytes as the most numerous category of bone cells is shown as the relative volume density in the control group and treated groups of animals in Figure 2. a as a percentage of the total volume.

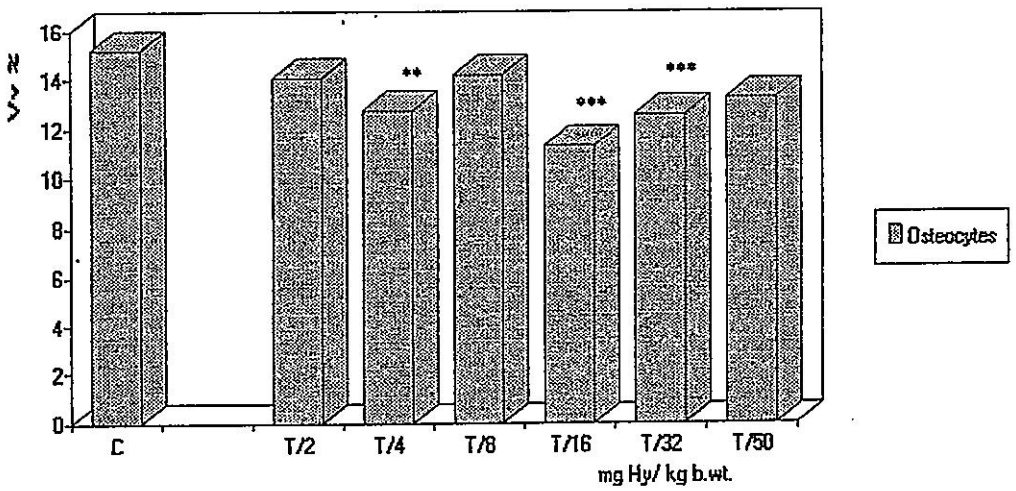

Figure 2a. Volume density (Vv) of osteocytes (oc) in control animals (C) and animals treated (T) with 2, 4, 8, 16, 32, and 50 mg Hy/kg of body weight.

Vv of osteocytes in the control group was 0,1520 mm^3 while the Vv of osteocytes in the treated groups was decreased. The differences between the control group and animals treated with 16 and 32 mg Hy were statistically highly significant ($p<0,001$). Thus values of Vv of osteocytes in animals treated with 16 and 32 mg Hy were 0,1127 mm^3 and o,1252 mm^3, respectively.

If we add those results to the previously obtained statistically significantly decreased values of numeric density of osteocytes in the same experimental groups in relation to the control one, there is an impression that hydrocortisone decreases the population of smaller osteocytes in comparison with the corresponding control group.

The finding that the volume density of osteocytes was decreased, as well as the numeric density, in the group treated with 4 mg Hy ($p<0,01$), in relation to the control group indicated that the starting concentration of hydrocortisone dausing a negative reaction is 4 mg Hy/kg of body weight.

On the other hand relative values for bone tissue matrix volume density were proportionally increased in the treated animals compared with the control ones (Figure 2 b). In this case the greatest differences were also noticed in the group of animals treated with 16 mg Hy ($p < 0,001$). The Vv value for bone tissue matrix in the control group was 0,8437 mm^3, and in the treated group it was 0,8872 mm^3. These values correlate with Vv values of osteocytes in the same group.

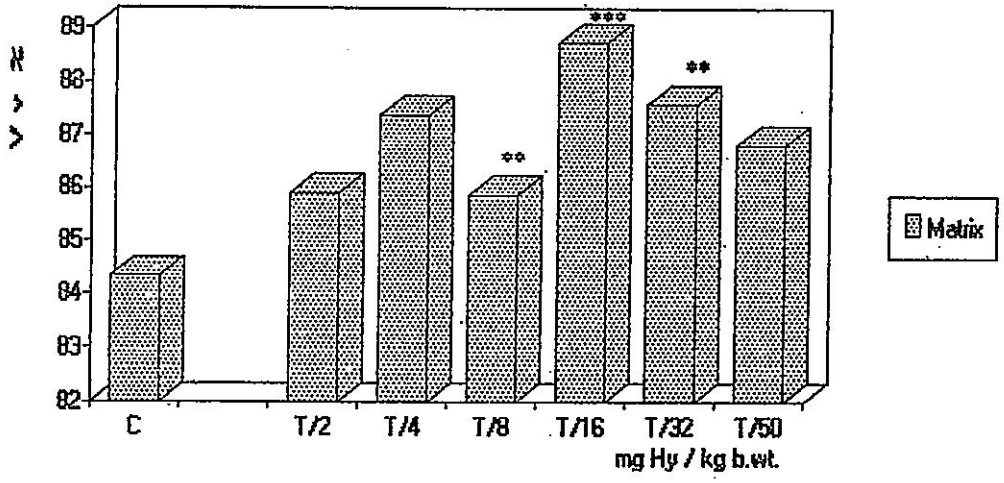

Figure 2 b. Volume density (Vv) of the matrix (m) in bone tissue in control animals (C) and animals treated (T) with 2, 4, 8, 16, 32 and 50 mg Hy/kg of body weight.

DISCUSSION

In mammals glucocorticoids may cause decreased formation of bone tissue and increased bone resorption, that is, they influence the metabolism of bone cells but the mechanisms of these effects are poorly defined (Jowsey and Riggs, 1970., Raisz et. al., 1972., Hahn et al., 1984.). Most research on glucocorticoid influence in clinical and experimental conditions concern changes in bone structure, as a result of long- duration hormone effect, and differences in the degree of reaction may be shown among groups treated with different doses (Raisz et al., 1972., Hahn et al., 1984., Chyn et al., 1984., Tenebaum and Herche 1985., Reginster et al., 1989.).

This was confirmed by the results of other authors who examined the influence of glucocorticoids on periodontal tissue. Thus, a low dose of 2,5 mg Hy given to rats of 130 g body weight did not cause any changes in the periodontium and femur in animals sacrificed after 10 and 20 days, but prolonged treatment for 30 days caused changes in bone tissue at both localities in 50% of the tested animals. There was distinct destruction of interdental periodontal tissue with a decreased alveolar crest (Blake and Zipkin, 1974). A dose of 0,5 mg Hy given to rats of the same body weight over a prolonged interval up to a year,did not cause any changes in the histological structure of the periodontal tissue, while a dose of 3 mg during the same time interval

caused an increase of alveolar bone matter, which could be the result of a decelerated process of endosteal resorption (Goldsmith and Stahl, 1953.).

Labelle and Schaffer, (1966), showed that in animals of 280 g body weight treated for five weeks with 2,5 mg of cortisone there was reduced cellular activity in periodontal bone, a distinct decrease of number and size of osteoblasts, and reduced formation of osteoid substance.

After 40 days of injecting 0,5 mg of cortisol a day to white mice of about 20 g body weight histological analysis showed osteoporosis of alveolar bone, distincitive by the decreased number of osteoblasts and quantity of newly formed bone matrix with reduced height of alveolar bone (Glickman, 1952., Glickman et al., 1952., Glickman and Shklar, 1955.).

The results of quantitative analysis in our study expressed through measuring the number of osteoblasts in mm^3 (Nv) showed that only supraphysiological doses of 16 and 32 mg Hy per kg of body weight, caused a decrease in the number of osteoblasts in the interradix septum in comparison with control groups ($p < 0,001$), when the animals were treated for 4 weeks. Doses of 2,4 and 8 mg Hy given to rats for the same prolonged period, seemed not to be sufficient to cause a reaction of the osteoblasts. Since the studies quoted from the literature are related mainly to quantitative analysis.

Some authors found the reasons for the decreased number of osteoblasts in the influence of Hy on preosteoblasts. Thus, Chun and coworkers (1984) examining early and later effects of cortisol on DNA and protein synthesis in periosteum and central bone in calvaria of the rat fetus in tissue culture found a significantly decreased incorporation of 3 H thymidine, as well as of radioactive proline, proportional to the dose of cortisol. At a high concentration of cortisol the number of mitoses in the periosteum was decreased, too. The number of cells was lower and the periosteum was thinner. The authors assumed that the primary influence of cortisol on bone growth was inhibition of proliferation of periosteum cells, that is, osteoblast precursor, which corresponds to previous results in vivo.

Osteocytes as a category were analyzed by two stereological parameters Vv and Nv which makes possible quantitative qualification of a given reaction. It seems that the degree of osteocyte sensitivity is directly linked to the hormone dose. Since the number and volume of osteocytes in the treated groups decreased compared with the control ones, and the differences were greatest in the groups treated with 16 and 32 mg Hy, it can be said that high Hy doses caused a very rare population of osteocytes, shown through Nv, and the osteocytes were of smaller volume. The smaller number of cellular elements in mm 3 maybe also caused by the decreased number of osteoblasts which produce osteocytes.However one possible mechanism of the Vv increase of the bone matrix could be decreased osteolysis of osteocytes. Glucocorticoids influence the metabolism of bone cells both in vitro and in vivo (Hahn et al., 1984., Raisz et al., 1972.), but the mechanisms of those effects are poorly defined. Thus, pharmacological doses of glucocorticoids decrease osteoblast activity and increase the activity of osteoclasts in vivo. These effects are shown

through direct or indirect mechanisms, and the latter includes an augmented effect of hormones through calcium and PTH (Bozovic and Devecerski, 1986.). It is considered that the moderate hypoglycemia caused by glucocorticoids leads to direct hypersecretion of parathyroid hormone (PTH) which stimulates osteolysis of osteocytes and resorption of bone by already existing osteoclasts. The production of new osteoclasts is stimulated as well. PTH releses collagenase and other enzymes that digest bone matrix. At the same time hydrocortisone obstructs the activity of osteoblasts and the formation of collagen. Corticosteroids inhibit active absorption of calcium and increase calcium secretion through the urine, which leads to hypocalcemia. In that way they directly stimulate the secretion of PTH, inhibit the influence of calcitonin and through these two hormones influence osteoclasts (Bozovic and Devecerski, 1986.).

According to our analysis a decreased number of osteoclasts was caused by doses of 16 and 32 mg Hy, while other doses were not sufficient to provoke a negative reaction. In some cases, such as treatment with 4 and 50 mg Hy, a weak increase of osteoclast number was noticed. The osteoclasts' reaction can be expressed with number and form of cellular elements, so in this other case the explanation of Nakamura and Kand (1983) seems acceptable. After analyzing osteoclasts and macrophages in enchondral bone of rat tibia after 15 days treatment with 30 mg Hy/kg, they found an increased number of osteoclasts in the zone of metaphyseal growth, but the frequency of osteoclasts from folded contours was low, and number of nuclei was decreased which indicates poor activity. In the region of metaphyseal trabeculae osteoclasts thinned. Differences in osteoclasts between the place of growth and the trabecular zone probably were connected with formation time of cell differentiation.

Osteoclasts are formed by coalescence of osteogenic cell precursors or by macrophage fusion. Glucocorticoids stimulate coalescence of osteogenic cell precursors into osteoclasts, but simultaneously inhibit production of osteogenic cells and macrophages. Since osteoclasts are short-lived (2-3 days), production of new ones will last until the existing supply of osteogenic cells is exhausted (Little, 1973.) which favors our results.

The observed decrease of osteoclast number after treatment with 16 and 32 mg Hy could be the result of death of already existing cells and decreased formation of new osteoclasts caused by the high doses and long - duration treatment of the animals.

These results are contrary to the results of Linck et al., (1968.), who found after injecting cortisone daily into young rats for six weeks a decreased depot of calcium in alveolar bone. They consider that this is the result of a two way hormone influence: namely inhibited activity of osteoblasts whose number is decreased so that cytologically they seem less active, and particularly the result of stimulated bone resorption through osteoclasts which became more numerous and cytologically more active. The authors did not register significant differences between doses of 0,25 mg and 40 mg cortisone per animal.

We suppose that the differences between their and our results come from differences in doses, in duration and way of treatment as well as from the different age of the animals. This assumption is in accordance with previously cited research.

CONCLUSION

According to our results it can be said that hydrocortisone has an influence on the diffusion of cellular elements and matrix of interradix jaw bone of rats treated for a long period with this hormone.

The decrease in number (Nv) of osteoblasts and osteocytes shows inhibition of osteogenic processes. This influence is proportional to the size of the dose and is significant at 16 and 32 mg Hy /kg of body weight.

The decrease in the number (Nv) of osteocytes and osteoclasts as well as the decrease of volume (Vv) of osteocytes in relation to bone matrix would favor inhibition of bone cleavage. It was noticed that 2 mg Hy/kg did not cause any significant changes in the number of osteoblasts and osteoclasts. Therefore, it could not be expected that in the limited time of the experiment, it could cause any change in the structure of interradix septum bone. The slightly changed percentage relation of Vv of bone matrix and Vv of osteocytes in the examined bone region is also in accordance with this.

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KOŠTANO TKIVO U INTERRADIKSNOM REGIONU U ŽIVOTINJA TRETIRANIH HIDROKORTIZONOM

SIMIĆ RUŽICA, ŠIMIĆ MARIJA, KARADŽOV OLGA i JOKIĆ NEVENKA

SADRŽAJ

Neposredan cilj ovoga rada je bio proučavanje efekta duže primene različitih doza hidrokortizona na koštane ćelije i matriks u interradiksnom predelu molara vilica u pacova tretiranih u periodu njihovog intenzivnog rasta. Na meziodistalnim presecima vilica vršena je stereološka analiza korišćenjem volumenske (Vv) i numeričke gustoće (Nv) vezivnih ćelija (osteoblasta, osteocita i osteoklasta) i volumenske gustoće matriksa koštanog tkiva.

Rezultati kvalitativne analize u našem radu, ukazuju da samo suprafiziološke doze od 16 i 32 mg Hy/kg tm, su uzrokovale pad vrednosti numeričke gustoće, za sve tri kategorije ćelijskih elemenata koštanog tkiva, sa visokim stepenom značajnosti razlika u odnosu na odgovarajuće kontrole. Osteociti, kao najbrojnija ćelijska kategorija, analizirani su i kroz parametar volumenske gustoće, pa kako je opao i broj i volumen osteocita u tretiranih grupa u odnosu na kontrole, a razlike su najizrazitije u grupama tretiranih sa 16 i 32 mg Hy, može se reći da su ovako velike doze Hy uzrokovale redu populaciju sitnijih osteocita. U skladu sa ovim je i nalaz neznatno izmenjenog procentualnog odnosa Vv koštanog matriksa, u ispitivanom regionu kosti vilica.