The histochemical effects of the administration of hydrocortisone sodium succinate upon the periodontium of albino rats' experimental study

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ABSTRACT

Background: The effects of the administration of cortisone upon numerous body tissues have been described; changes in the periodontal tissue of developing albino rats (newborn) histochemically have not been described. A study was therefore, under taken to investigate the effect of administration of cortisone upon the periodontal ligament tissues.

Materials and methods: Experimental animals (new born rats, their age 10, 19, 25 days old) were treated by cortisone, saggital sections were stained by alcian blue and H&E stains.

Results: It was shown that the hydrocortisone sodium succinate affect that periodontal tissue development and this drug inhibit and delay the synthesise of GAGs (glycosaminoglycans) till aged 25 post natal (p.n.).

Conclusion: The periodontal membrane in the treated groups is affected (delay in the GAGs synthesis) by the hydrocortisone sodium succinate drug administration.

Keywords: Hydrocortisone, periodontal ligament, alcian blue. **(J Coll Dentistry 2005; 17(2):59-63)**

INTRODUCTION

Corticosteroids is produced by the adrenal cortex, and are divided into natural and synthetic cortical steroids, hydrocortisone sodium succinate (cortisol) is predominant natural gluco corticold in human. [(1)]

It is indicated for the treatment of rheumatic disorders, asthma, collagen disease, dermatological disease, heamatologic disorders. The adverse reactions include fluid and electrolyte disturbances, musclo skeletal disorders, metabolic imbalance, endocrine irregularities and long term cortiosoteroids use may be associated with more serious sequal including osteoporosis, growth suppression, possible congenital malformations [(2,3,4)]. The osseous changes consisting of reduction in chondrogenesis and osteogenesis in the proximal epiphyseal cartilage, reduction in numbers of large vacuolated cartilage cells, reduction in number of osteoblast, irregular bony trabeculae and wider marrow sinusoids were described in experimental animals. [(5)] histomorphological changes (histological abnormalities) in the periodontal ligament Periodontal ligament. of developing tissue that treated by cortisone have been described by many authors[6,7]. However, there is no previous report remark alternate in their histochemical changes so this study was done to know the effect of hydrocortisone sodium succinate on Periodontal ligament development histochemically (by using alcian blue stain).

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MATERIALS AND METHODS

Thirty pregnant rats were randomly divided into two main groups: the control (A) and the treated groups (B).

A. <u>The control groups</u>

This group consisted of 15 pregnant rats, each one was given 1ml distilled water by i.m. (intramuscular) injection daily from (12 to 18) day of pregnancy. After delivery of the pregnant rats, 4 newborn rats were selected randomly to be sacrificed at 10, 19 and 25 days old.

B. <u>The treated group</u>

This group consisted of 15 pregnant rats, each rat given a dose of 50 mg/ml hydrocortisone succinate by i.m injection, in lateral left thigh once daily from 12 to 18 day of pregnancy, hydrocortisone succinate was suspended in normal saline to obtain concentration of 100mg/2ml, and 1ml of volume was given i.m each time. After delivery of the treated pregnant rats by cortisone, roughly 4 new born rats were selected to be sacrificed at 10,19 and 25 days old (table 1).

Table 1: The number of newborn rats in the experimental design

days old / Groups	10	19	25
Control group	20	20	20
Treated group	20	20	20

<u>The General Histological Preparation.</u>

The head was separated and cut saggitally into 2 halves; both sides were used in the control and experimental groups. After fixation,

decalcification, dehydration, clearing and embedding, mid saggital section of the specimens were cut at 5 microns by Reichert Jung microtome, one of each sections is stained with Harris Hematoxylin and eosin (H & E) and the other is stained by alcian blue (see below). All the sections were examined by Olympus light microscope.

The special stain (Alcian blue) histochemistry.

The cationic dyes (special stain is alcian – blue prepared at pH1 and pH 2.5 pathalo cyanine dyes) are derivative of copper phthalocyanin and appear as a blue pigment of high physical and chemical stability,[8] it is used in this study.

First concentration, alcian blue pH 2.5

1% alcian blue solution was used for staining of weakly acidic mucopolysaccharides. It was prepared from mixing Alcina blue 8 GX I.g with glacialacetic acid 3% 100ml then filtered; it remains stable 2–4 weeks.

Second concentration, alcian blue pH 1

1% alcian blue solution was used for more selective staining of strongly acidic sulfated mucosubstances. It is as well consisted from mixing

Alcian blue 8Gx 1.g was used with hydrochloric acid 0.1n. (1% HCl) 100mL and then filtered before use. Beside that a counter stain (Kernechtrot solution) was used. The slides were washed in running water (1min) and dehydrated, cleared and mounted [(9,10)].

RESULTS

Gross observations

The cortisone treated animals appeared less active and smaller than the control at the time of sacrifice, while the gingiva of both groups showed marginal inflammation associated with local irritation from food particles.

The destruction of the inter dental papilla appeared to be more sever in cortisone treated animals

Microscopic Observation.

The interdental bone between the molars teeth was lined on one aspect by a thin layer of osteoid with adjacent osteoblast (Fig 1and 3). Along the other aspect, the bony border was smooth in some areas and irregularly in others. The multinuclear osteoclast was seen in concavities along the surface at the gingival cryst of the bone, a small section of osteoid was seen, and the periodontal membrane was densely collagenous with well formed fibroblasts.

While in the cortisone treated animals the interdental septa were reduced in height, the margins of the septa stained deeply and presented a minutely granular border, there was no appreciable evidence of osteoblast or osteoblastic activity, in a few isolated areas, shrunken, deeply staining osteoblasts were seen partially surrounded by faintly staining osteoid. The appositional lines were less clearly marked and in some instance were unusually irregular, the periodontal membrane was edematous, the fibroblasts were markedly reduced in number and shrunken in appearance, and the collagen fibers were reduced in amount, wavy and fibrin like and separated by the edema. (Fig.2 and 4)

In the bifurcation area of the molars, a broad peripheral zone of newly formed bone was seen adjacent to the periodontal membrane, demarcated from the under lying bone by deeply staining appositional line , osteoblasts were aligned along the periphery, separated from each other by collagenous fibers embedded in the bone. The periodontal membrane consisted of densely arranged fibers with numerous well formed cells. (Fig. 5). While The bone was outlined by a deeply staining irregularly indented border with the exception of a small area of new bone formation and a few poorly staining osteoblasts at the crest, of the treated animals. There was no evidence of peripheral bone apposition, the periodontal membrane was edematous, and the fibroblast were markedly reduced in number and shrunken in appearance. The collagen fibers were reduced in amount wavy and fibrin like and separated by edema. (Fig. 6).

The cementum was thin and acellular in the gingival half of the roots and bulbous and cellular in the apical region of the control animal, While the cementum of treated molar teeth appeared thinned and devoid of newly formed cementoid (Fig 5,6).

Glycosaminoglycans (Hyaluronic acid, chondroitin sulphated and heparin sulphate) Histochemistry.

The reaction in general was stronger at PH 2.5 than PH 1, indicating the presence of both sulfated and non sulfated (Carboxylated) glycos aminoglycans. For hyaluronic acid and sialic acid as revealed by alcian blue PH2.5:

In the control groups the reaction was strong at 10 p.n, weaker at 19, 25 p.n Fig (7). While, In the treated groups, The reaction was

stronger than the control in the treated groups (19 p.n, 25p.n) Fig (8), while for chondroitin sulphate and heparin sulphate as revealed by alcian Blue (PHI). In the control group, there was strong reaction at 10 Pn, but it becomes weak at 19, 25Pn Fig (9). While, in the treated groups there was intense reaction at 19, 25Pn Fig. 10.

DISCUSSION

The treated group gives obvious histological and histochemical abnormalities in their developing periodontal ligament, indicating alteration in normal developmental processes. It is well established that cortisol, cross the placenta [(11)]. All the body compartments of fetus are affected by cortisone reaching them via fetal blood carrying it from their treated mothers.

Hydrocortisone inhibits protein and glycoprotein synthesis, decreases amount of proliferating cells, accelerates their differentiation and thus delay the development process, this was reported by Mazhuga et al.[(12)] It should be noted that bone and periosteum in other area of the skeletal system presented changes [(4)] similar to those in the alveolar bone and periodontal membrane.

This inter relationship is consistent with the often repeated observation in experimental animals that the alveolar bone and periodontal membrane reflect the condition of the remainder of the skeletal system in systemically induced osseous disturbances [(13)].

In the periodontal membrane of cortisone injected animals the alterations in the fibroblast and collagen fibers are comparable to those described in connective tissues of other experimental animals, and human beings subjected to systemic administration of cortisone [(14,15)]. The periodontal membrane at age 10 Pn is intensely stained by alcian blue so this indicate the presence of proteoglycan (GAG) in that membrane but when developing continues the staining become less or removed indicating the very little amount of proteoglycan in that membrane [(16)]. In well developed P.d ligament structure (at 19, 25Pn) the presence of GAGs in 10p.n during P.d. ligament development have shown close association of these proteins with different stages of cell differentiation (Mast cells, fibroblast cells) and/ or in cell secretion in the ground substance. In the treated groups the weak reaction at 10 Pn indicating that hydrocortisone drug inhibits the synthesis of GAGs. But when time pass and the new born rat become older at 19 p.n and 25p.n the effect of this drug is reduced and the P.d. ligament become normal in its content with GAGs (Chondroitin sulphate, heparinsulphate, hyaluronic acid) so there is delay in the formation of proteoglycan content at P.d. ligament because the reactivity is still until the age of 25 p.n is under the effect of the drug.

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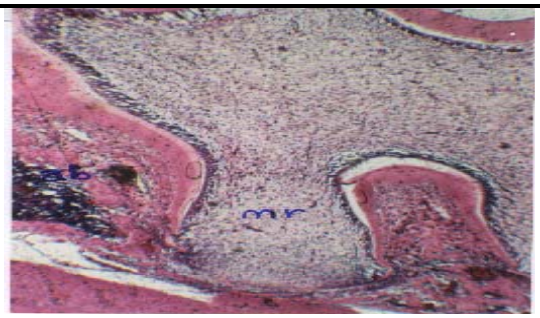

Figure 1: Control group, a mesiodistal section through the mandibular molar area showing molar roots (m.r) and alveolar bone (a.b) (H & E x 40).

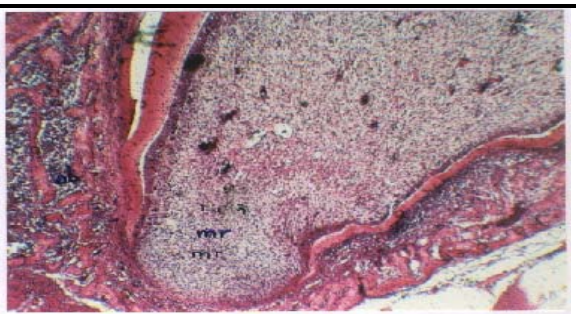

Figure 2: Experimental group; a section through the mandibular molar area showing molar root (mr) and alveolar bone (a.b) (H & E x 60).

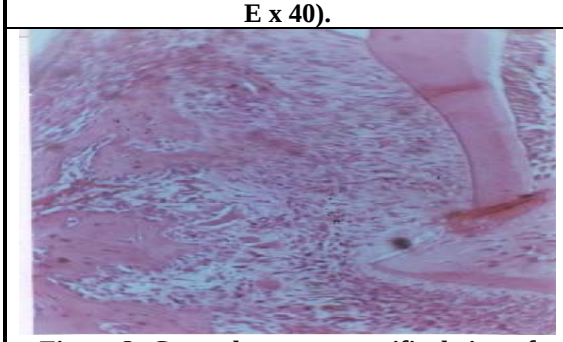

Figure 3: Control group, magnifical view of interdental septum and periodontal membranes (p.m) shown in fig 1. There are osteoblasts and osteoclast in regular sence. (H & E x 250).

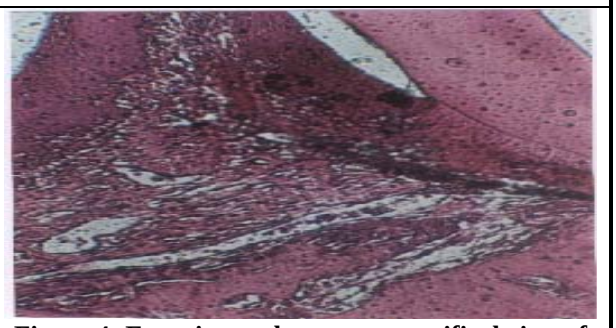

Figure 4: Experimental groups, magnifical view of inter dental septum and periodontal membrane shown in fig. 2. Note irregularly sence of osteoblasts and osteoid. The fibroblast of periodontal membrane are reduced in number and the collagen fibers appear fibrin like and fragmented (H & E x 250).

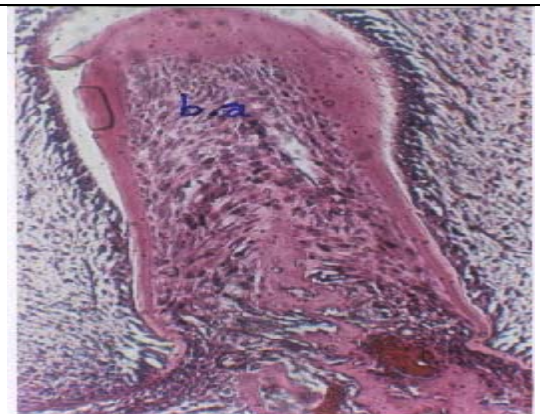

Figure 5: Control group, detailed section of bifurcation area (b.a) of the molar shown in fig 1. note the broad peripheral zone of recently formed bone which is demarcated from the underlying bone by a deeply staining appositional line and normal thin cementum (H & E x 250).

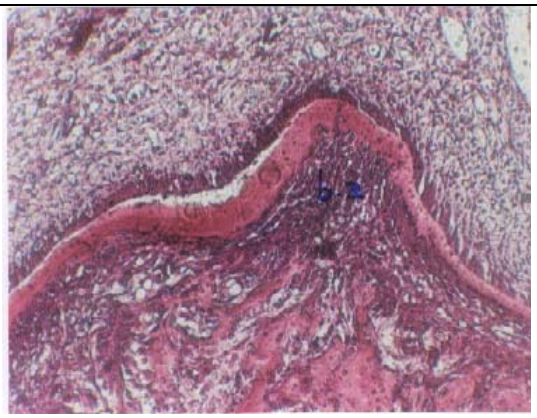

Figure 6: Treated group – Detailed study of bifurcation area (b.a) of the molar shown in fig. 2. A side from a small area of osteoid at the crest, there is no apposition of newly formed bone. In stead the margin of the bone is formed by a thin deeply staining irregularly indented line. Note the very thin cementum (H & E x 250).

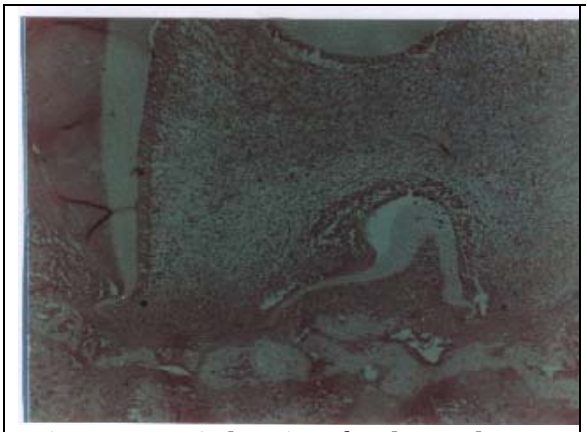

Figure 7: Saggital section of molar tooth at 19 p.n in the control group stained with alcian blue pH2.5. Note: The reaction is weaker at periodontal ligament area

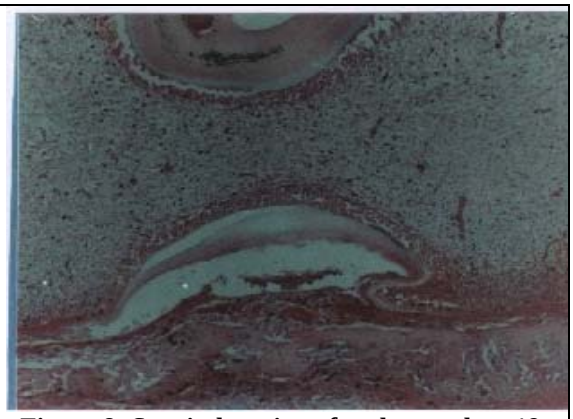

Figure 8: Saggital section of molar tooth at 19 p.n in the treated group stained with alcian blue pH2.5.
Note: The reaction is more intense at periodontal ligament area