Healing of Tooth Extraction Wounds in Rats with Renal Hypertension. A Histological Study

by

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Summary

The aim was to study bone formation in tooth extraction sockets of rats with experimentally induced renal hypertension. As soon as hypertension was detected the upper right incisor was extracted. The animals were killed at 3, 6, 9, 12, 18 and 21 days post-extraction. At day 9 the hypertensive animals showed bone formation with little osteoblastic activity, the bone was more cellular and had immature osteoblasts and endosteal cells without trabeculae formation. In the medullary spaces oedematous connective tissue, dilated vessels and undifferentiated cells were seen. We concluded that these animals presented a different pattern of bone formation when compared to the control animals.

Key words: Bone wound healing. Tooth extraction. Experimental hypertension. Rat renal hypertension. Socket histological study.

Introduction

Experimental renal hypertension has been studied since GOLDBLATT et al.[8] (1934). There have been some modifications in their methodology and in the use of various other animal species.

The cause of hypertension in the Goldblatt models has been identified as a retention of sodium and water in the animal body (LIARD and PETERS[11], 1973). However, the increase of arterial pressure in these animals, in the initial stages, can be attributed to the renin-angiotensin system (CARRETERO and ROMERO[3], 1977).

CASTELLI et al.[4] (1978) studied the effects of high arterial pressure in vessels supplying the gingiva, periodontal ligament, pulp tissue and alveolar bone in rats with renovascular hypertension. They found that the most significant alterations in the gingival vessels were thickening, and noted hyalinization of the vessel walls with narrowing of the lumen and proliferation of the elastic lamina.

The effect of experimental systemic alterations on the healing process of tooth extraction wounds has provided information about the chronology of alveolar repair. Repair in extraction wounds begins with the formation of a blood clot, followed by replacement by granulation tissue and then osteoid tissue from the apical portion and the lateral walls of the alveolus. The osteoid tissue is gradually turned into mature bone. The formation of epithelial tissue on the wound surface is completed during

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the early-post-operative stage (Euler[7], 1923; Claflin[6], 1936; Christopher[5], 1942; Amler et al[1], 1960; Okamoto and Russo[13], 1973).

In one of the few papers studying hypertension and healing of dental extraction wounds, Murata et al.[12] (1967) reported that the healing process was slower than in normal dogs and that the degree of retardation was proportional to the degree of hypertension. The object of this study, therefore, was to investigate further the influence of experimentally induced hypertension on the healing of extraction wounds.

**Materials and Methods**

Forty-eight male rats (Rattus norvegicus, albinus, Wistar) weighing 150 to 200 grams were used. They were kept in plastic cages (4 rats per cage), fed on solid commercial food (Anderson Clayton, Brazil) and received water ad libitum. These animals were divided into two equal groups, one control normotensive and the other hypertensive.

The animals were anaesthetized with sodium pentobarbital (45 mg/kg body weight) administered intraperitoneally.

Renal hypertension was induced by constriction of the left renal artery using an omega shaped silver clip. The contralateral kidney was removed according to a technique of Goldblatt et al.[8] (1934) and adapted by Schaffenburg[16] (1959). The internal diameter of the clip was based on animal weight: rats of 150 g = 0.35 mm to rats of 200 g = 0.40 mm. The abdominal wound was sutured with 3-0 silk (Johnson & Johnson, Brazil). The rats received approximately 80,000 IU of antibiotic (Pentabiotico, Fontoura Wyeth, Brazil).

Arterial pressure was recorded indirectly from the 15th post-operative day on by tail plethismography in the unanaesthetized rats as described by Williams, Harrison and Grollman[18] (1939). The animals were considered to be hypertensive when their blood pressure exceeded 135 mmHg (Krieger[10], 1964).

The animals were anaesthetized with sodium pentobarbital (45 mg/kg body weight) administered intraperitoneally and the upper right incisors were extracted by means of an instrument specially designed by Okamoto and Russo[13] (1973). Following extraction the socket was sutured with 3-0 silk (Johnson & Johnson, Brazil). Four animals from each group were killed by ether anaesthesia at 3, 6, 9, 12, 18 and 21 days post-operatively. The upper right maxilla was removed and the excess tissue was carefully excised leaving the socket intact. The jaws were placed immediately into 10% neutral formalin and when fixed they were demineralized in 20% sodium citrate and 50% formic acid solution, mixed in equal parts. Buccolingual serial sections, 6 micrometers thick, were stained with Harris haematoxilin and eosin.

**Histological Results**

For the description of the results the socket was divided into cervical, middle and apical thirds.

3 days—In the control group the socket was filled with a blood clot. The buccal alveolar crest showed signs of osteoclastic activity. The cervical third contained a fibrin network infiltrated by moderate numbers of polymorphonuclear neutrophils.
At the middle and apical thirds, adjacent to the cortical wall, young fibroblasts had migrated into the clot from remnants of the periodontal ligament (Fig. 1). Osteoblastic proliferation with osteoid deposition from the cortical wall was observed.

In the hypertensive group, features similar to those of the control group were seen but included bone deposition at the middle and apical portions.

6 days—In the control group, a blood clot was observed in the socket. The buccal alveolar crest showed bone apposition on the external surface only (Fig. 2). The cervical third showed granulation tissue formation (Fig. 3). In the middle and apical thirds granulation tissue and bone formation were seen in some areas.

In the hypertensive group, a blood clot was observed in the socket. The buccal alveolar crest showed bone apposition on the external surface. The cervical third revealed granulation tissue in the blood clot and in the middle and apical thirds there was intensely cellular granulation tissue with hyperaemis of the newly formed vessels (Fig. 4).

9 days—In the control group the alveolar crest was remodelled. At the cervical third, bone trabeculae and larger medullary spaces with more intense cellular activity were observed. The middle and apical thirds were almost filled with newly formed bone having immature central trabeculae.

In the hypertensive group the buccal alveolar crest was remodelled with immature bone. The cervical third presented little osteoblastic activity and bone formation with characteristics of immaturity. They were more cellular, with immature osteoblasts and endosteal cells without trabeculae formation. In the medullary spaces oedematous connective tissue, dilated vessels and undifferentiated cells were seen.

12 days—In the control group the cervical third of the socket contained bone trabeculae adjacent to the cortical wall. The middle and apical thirds had compact bony trabeculae near the cortical wall and was less compact in the central portion (Fig. 5).
The areas not filled up by bone showed well differentiated connective tissue.

In the hypertensive group all the parts of the socket presented trabecular formation with characteristics similar to day 9 (Fig. 6), with the central portions containing granulation tissue. The areas not filled by bony tissue also appeared to be similar to the features seen in day 9.

18 and 21 days—In the control group the whole socket contained mature and compact bone trabeculae and reduced medullary spaces with discrete osteoblastic activity. Bone formation was regular with basophilic resting and reversal lines accompanying the morphology of trabecular formation (Fig. 7).

In the hypertensive group the whole socket contained immature trabeculae. These sometimes appeared without resting and reversal lines, but when they were present they did not accompany trabecular formation (Fig. 8).

**Arterial Pressure Results**

<table>
<thead>
<tr>
<th>DPE</th>
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<th>6</th>
<th>9</th>
<th>12</th>
<th>18</th>
<th>21</th>
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<tr>
<td>BP (mmHg)</td>
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<td>166.25</td>
<td>162.75</td>
<td>183.75</td>
<td>165.50</td>
<td>173.75</td>
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<td></td>
<td>±7.82</td>
<td>±11.43</td>
<td>±12.08</td>
<td>±12.48</td>
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**Discussion**

In the model of hypertension used in this experiment the severity is proportional to the degree of renal artery constriction with the blood pressure increasing rapidly and remaining elevated for years (CARRETERO and ROMERO[3], 1977).

The health of the remnants of the periodontal ligament remaining after tooth extraction is important as it is from here that fibroblastic proliferation begins. The descriptions of JOHANSEN and GILHUUS-MOE[9] (1969), OKAMOTO and RUSSO[13] (1973) taken with our results clearly confirm these aspects.

Our results showed bone formation only on the external surface of the buccal alveolar crest of the socket during the early post-operative stages, which is in agreement with the observations of BOYNE and KRUGER[2] (1962) and JOHANSEN and GILHUUS-MOE[9] (1969). According to these investigators this bone formation is thought to be a reinforcement of the alveolar process and possibly acts as a compensatory reaction to the change in function to which the bone is submitted.

The kidney is the site where the vital hydroxylation of vitamin D occurs. Renal failure limits this function and animals with such a chronic disturbance frequently develop signs and symptoms of vitamin D deficiency, as stated by ROBBINS[15], 1975 and VAUGHAN[17], 1975. It is possible that vitamin D, in its more active form, may act on the bone matrix and on osteoblastic activity (VAUGHAN[17], 1975). In a vitamin D deficiency, with an inadequate deposition of calcium salts in the organic bone matrix, there is insufficient mineralization, excessive formation of osteoids and
development of irregular bone, similar to that observed in this study.

At 18 and 21 days post-operatively the trabecular pattern of the formed bone in the sockets of the hypertensive rats was not normal due to the immature character of the trabeculae, suggesting an interference in mineralization. It is possible, therefore, that hypertension has a systemic effect that alters the reabsorption and hydroxylation of vitamin D and that this interference consequently alters bone repair of the sockets.

By studying the healing of dental extraction wounds in dogs with hypertension experimentally induced by the method of Page[14] (1930) and Murata et al.[12] (1967) showed delayed healing. This alteration was directly related to the degree of hypertension, and not to its duration. They also found intense bone formation at 10 and 20 days post-operatively, but at the end of 56 post-extraction days, repair was not complete.

Conclusions

The present results show that experimentally induced hypertension alters bone repair in dental extraction wounds of rats and changes the pattern of bone formation which is different from the control animals because of its immaturity.

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References


