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Periapical Lesion Development in Rats Inhibited by Dexamethasone

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Bone resorption is one of the hallmarks of inflammatory periapical lesions and is mediated by cytokines. Recent insights into the immunobiology of these lesions indicate that pharmacological modulation of their bone resorbing activity may be possible. Periapical lesions were induced in rats by occlusal exposure of the pulps of their lower first molars. The size of the resulting lesions was followed-up and evaluated by image analysis of their radiographs. The lesions increased with time, and the average area of their radiographic images reached 2.18 (±0.33) mm^2 by day 21. Systemic dexamethasone treatment (0.5 mg/kg, every third day) inhibited the growth of the periapical lesions, which reached an average area of 1.63 (±0.30) ($p < 0.01$). These results support the hypothesis that bone resorption in periapical inflammatory lesions may be pharmacologically down regulated.

Inflammatory periapical lesions develop in response to bacterial contamination of the root canal. Bone resorption is one of the hallmarks of this type of lesion, and its radiographic image is one of the major clinical tools by which its development or resolution is monitored and evaluated.

During the last decade, major advances were made in the understanding of the immunobiology of bone resorption in apical periodontitis by using the rat model (1, 2). Locally produced interleukin (IL)-1α and to a lesser extent tumor necrosis factor (TNF)-α and IL-1β are the major inflammatory mediators that cause the periapical bone resorption in these animals (1). In humans, IL-1β and TNF-β are the major cytokines involved in this process (3). The main potential sources of these cytokines are activated macrophages and activated T-lymphocytes (4, 5). Nevertheless, the recent demonstration that athymic rats and mice, which lack T-lymphocytes, develop periapical lesions at the same rate as normal animals indicates that macrophages are the key players in this process (2, 6).

Macrophage activation may be induced in these lesions by either a T-cell mediated process or directly by bacterial components, such as bacterial endotoxin (LPS). The former activation pathway involves the production of interferon γ (INFγ) by antigen activated T-lymphocytes, whereas the latter is independent of the T-cells.

The traditional approach to the prevention and treatment of periapical inflammatory lesions centers on the prevention of bacterial contamination of the root canal or eliminating the bacteria from the infected canal and preventing its recontamination. Once this goal has been achieved, healing of the lesions is commonly followed-up radiologically with no further attempt to modulate the process. The recent knowledge of the cells and mediators that are involved in periapical bone resorption may lead to a new rationale of treatment (7). Following, and in addition to, the traditional approach, pharmacological modulation of cytokine activity in the lesion may be considered to down-regulate bone resorption and thus enhance the resolution of the lesion.

Stashenko et al. (8) have demonstrated that such intervention may be possible. Their target was the effect of the locally produced IL-1. By treating the animals with IL-1 receptor-antagonist, they were able to prevent some of the effects of this locally produced cytokine, which resulted in a significant reduction in the size of periapical lesions (8).

In this study, we targeted another link of the chain of events leading to bone resorption—the activation of macrophages. It has been shown that steroids may turn off macrophage activation by inhibiting the production of their inflammatory cytokines at the transcriptional level (9, 10). Steroids were also recently used to inhibit the suppressive effect of activated macrophages on fibroblast growth (11). We, therefore, studied the effect of dexamethasone on the development of periapical lesions in rats to demonstrate that this may be another possible route for pharmacological modulation of the bone-resorbing activity in these lesions.

MATERIALS AND METHODS

Experimental Design

Periapical lesions were induced in rats, as described by Stashenko et al. (12). Their development was followed for 14 or 21 days. The effect of systemic administration of dexamethasone was evaluated by comparing lesion size in the treated animals to that of control animals that were sham-injected with saline.

Animals

Four-month-old, female, Sprague Dawley rats (Animal Research Center, Bar Ilan University, Ramat Gan, Israel) were used

in the experiments. Their average weight was 340 g (± 13) at the beginning of the study. The rats were kept in separate cages and were fed pelleted rat diet, ad libitum. Each rat was weighed every third day, and weight gain of the dexamethasone-treated group was recorded and compared with that of the control group and of normal animals, upon which no procedure was performed.

Pulp Exposure

Animals were anesthetized by intra-peritoneal injection of 90 mg/kg of ketamine. The pulps of their two lower first molars were exposed, using a #0.5 round bur, which penetrated to a depth equal to the diameter of its active part. The pulps were left exposed to the oral environment for the duration of the experiment.

Dexamethasone

Dexamethasone (Dexacort, Teva, Israel) was dissolved in saline and injected intramuscularly (0.5 mg/kg) every third day, starting 48 h before the pulp exposure. Animals in the control group were sham-injected with saline at the same time intervals.

Radiography

At 14 or 21 days, animals were killed, their mandibles removed, and placed for 48 h in 2% NaOH, to allow a thorough removal of soft tissues and separation into right and left parts. The jaws were radiographed on a Type M, ultra-fine grain industrial film (Kodak Industrial X-ray Film, Eastman Kodak Co. Rochester, NY). A 2-s exposure was used from a distance of 25 cm, using a Philips Secodent E X-ray machine (Philips, Monsa, Italy). Uniform positioning of the jaws was achieved by using a mold made of dental impression material, which held their posterior part and positioned each of them at the same angulation to the film.

Image Analysis

The radiographs were scanned by using a HP Photosmart film scanner (Hewlette Packard, Singapore), and their digital image was analyzed by using Sigma Scan software (SPSS Science Software, San Raffael, CA). The borders of the radiographic image of each periapical lesion were traced and its area calculated. In each animal, a total of four periapical lesions were measured, two (mesial and distal) for each first lower molar.

Statistical Analysis

An average area of the periapical lesions was calculated for each group (±SEM). The groups were compared to each other by using Student's *t* test.

RESULTS

Animal Weight and Health

The weight of the sham-injected, control animals gradually increased from an average of 346 (± 13) to 440 g (± 15) by day 21. Their weight gain did not differ from that of normal animals, upon which no procedure was performed. The weight of the dexamethasone treated animals decreased by 6% after the steroid administration was started and stayed unchanged through day 21. None of the dexamethasone treated animals developed any clinical signs of a local abscess or of spreading infection.

Periapical Lesions in Control Animals

The periapical lesions that developed in response to pulp exposure gradually increased with time. At 14 days, the average area of their radiographic image was 1.62 mm^2 (± 0.45) and it reached 2.18 mm^2 (± 0.33) by day 21.

The Effect of Dexamethasone on Periapical Lesion Size

Systemic dexamethasone had an inhibitory effect on the development of the periapical lesions. By 21 days, the average area of the lesions' radiographic images was 1.63 mm^2 (± 0.30) in the dexamethasone treated group, which was significantly lower than the size of the lesions in the control group: 2.18 mm^2 (± 0.33) ($p = 0.008$).

DISCUSSION

Apical periodontitis is an expression of a defensive host response that prevents the spread of infection from the contaminated root canal to the surrounding bone. Therefore, the use of steroids to modulate this inflammatory response has been controversial. Those who oppose it worry that it might jeopardize the patient by down-regulating the host response in this site. Taking this approach, one could expect that animals with infected root canals, which were treated with a high systemic dose of dexamethasone, would develop larger uncontrolled lesions or even suffer from spreading infection. None of this occurred. None of the animals developed any clinical signs of systemic infection or even a clinically evident local acute abscess. On the contrary, the size of the lesions in the dexamethasone-treated group was smaller than in the control.

Dexamethasone was used in this study at a dose that is equivalent to a short-term high dose used in humans. Administration of the steroid has a gastrointestinal side effect of malabsorption and resulted in the inhibition of weight gain in the treated animals. The dental procedure alone (pulp exposure on the occlusal surface of the first lower molar) had most probably almost no effect on the eating habits of the animals, which was evident from the normal weight gain of the control group.

Long-term, systemic, dexamethasone administration is commonly associated with reduction in total bone mass, which is frequently interpreted as direct enhancement of bone-resorbing activity (13). Nevertheless, this effect seems to be related, in part, to diminished intestinal calcium absorption rather than a direct effect on the osteoclasts. Reduced intestinal calcium uptake results in lowering calcium blood levels, which in turn trigger PTH secretion that leads to the systemic bone resorption observed (14).

The inhibitory effect of the steroid on periapical bone resorption could be mediated mainly by two targets, the activated macrophages or the osteoclasts. Dexamethasone inhibits production of inflammatory cytokines by LPS-activated macrophages at the transcriptional level (9, 10). This mechanism alone could explain the observed inhibition of lesion size development. Nevertheless, the

osteoclasts themselves may also be affected by the steroid. Enhanced apoptosis of osteoclasts was observed when exposed to steroids, thus reducing their numbers (15, 16). Steroids may also up-regulate the expression of calcitonin receptors on osteoclasts, making them more responsive to the normal serum levels of this hormone (17). This, in turn, may lower their bone-resorbing activity.

It should be noted that in this study no attempt was made to decontaminate the root canals, and the effect of the steroid was clear, even when the bacterial activating stimuli persisted. It may be assumed that if bacteria were first eliminated from the root canals, this effect may have been more pronounced. Our findings should not be interpreted, at the present stage, as a recommended clinical protocol but rather as pointing out the potential for pharmacological modulation of the bone-resorbing activity in periapical lesions. The steroid was used systemically due to the size limitation of the rat molar. Nevertheless, in humans, materials of a modulating potential may readily be applied as local agents, rather than used systemically.

In the future, local modulation of periapical host response, aimed to enhance its resolution, may supplement the traditional treatment protocol. Once the root canal has been decontaminated, a slow release device may be placed in it. It may be shaped as a point, which will gradually release the selected agent through the patent apical foramen for a desired period of time. Several groups of candidate materials may be considered for local modulation of bone resorbing activity. These include agents that may be targeted at (a) the production of the bone-resorbing cytokines and their release; (b) preventing the interaction of these cytokines with the osteoclasts; or (c) direct modulation of the generation and/or activation of the osteoclasts. The first group may include steroids, as suggested by our results, as well as tetracyclines, which inhibit the release of already formed inflammatory cytokines from the activated macrophages (18). The second may include IL-1 receptor-antagonist as suggested by Stashenko et al. (8). The third group may include the steroids, as discussed above, as well as two applications that emerge from the recent advances made in osteoporosis research. One may use bisphosphonates, such as alendronate, which may locally inhibit bone resorption (19). The second may use the recently discovered osteoprotegerin, which has been defined as the natural down-regulating agent for both osteoclast generation and the activation of existing osteoclasts (20).

The concept of pharmacological down regulation of periapical bone resorption may lead to a major breakthrough in enhancing the healing of inflammatory periapical lesions. Nevertheless, intensive research will be required before it may be clinically applied.

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