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The effect of intracanal anti-inflammatory medicaments on external root resorption of replanted dog teeth after extended extra-oral dry time

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Abstract – The prognosis of replanted teeth depends on several factors, the most important being the length of extra-oral dry time. Studies show that after 60 min dry time, root resorption is predicted. Immediate intracanal placement of Ledermix, a paste containing triamcinolone (corticosteroid) and demeclocycline (tetracycline) has been shown to inhibit root resorption after extended dry time. However, discoloration is possible from the tetracycline in Ledermix. To evaluate the individual influence of corticosteroid and tetracycline on external root resorption after extended extra-oral dry time. Sixty-nine premolar roots of four beagle dogs were extracted and instrumented with NiTi files. Group 1 (negative control) was immediately replanted after root filling with GP and sealer; Group 2 (positive control) was root filled with GP and sealer and replanted after 60 min dry time; In groups 3–5, the canals were filled with Ledermix, Triamcinolone, and Demeclocycline, respectively, and replanted after 60 min dry time. After 4 months, the dogs were sacrificed and the roots were examined histologically for type of healing (favorable or unfavorable) and remaining root diameter. The groups treated with Ledermix, Triamcinolone and Demeclocycline had statistically significantly more favorable healing (75.8%; 69.8%; 52.4%) and more remaining root structure (5.59; 5.48; 5.09) than the group filled with GP and sealer (positive control) (0; 1.15). Corticosteroids were as effective as Ledermix at inhibiting external root resorption.

The prognosis of replanted teeth depends on several factors, the most important being the length of extra-oral dry time (1, 2). Studies show that after 60 min dry time, the viability of the periodontal ligament cells is almost zero, thus external root resorption is predicted (2). The first reaction after replantation of a tooth with a damaged root surface is inflammation, which includes osteoclast-mediated root resorption in proportion to the initial damage (3).

If the initial damage is small, as when an avulsed tooth is replanted immediately, and no additional inflammatory stimulus is present, the damaged root surface will heal with cementum (Favorable healing) (4, 5). On the other hand, if the damaged area is large, or additional inflammatory stimulus is present as after extended dry time, direct bone to root fusion (ankylosis) or osseous replacement will take place (4, 5). Thus, if we can minimize or shut down the initial inflammatory response, we will minimize the size of the damaged root and therefore promote favorable (rather than unfavorable) healing (3).

Ledermix^{TM} (Lederle Pharmaceuticals, Wolfrantshausen, Germany) is a water soluble paste containing 1% Triamcinolone (Corticosteroid) and 3% Demeclocycline (Tetracycline). Both Triamcinolone and Demeclocycline have anti-resorptive properties. Previous studies have demonstrated the diffusivity of these two anti-inflammatory agents placed in the root canal space via the dentinal tubules to the root surface for up to 14 weeks, in which the rate of diffusion was significantly higher for all time periods when cementum was devoid as a result of trauma (6–8). Ledermix has also been shown to significantly reduce root resorption after extended dry time extensively because of its anti-inflammatory properties (9, 10). However, a major clinical concern is possible discoloration which occurs because of the tetracycline in Ledermix (11).

The purpose of this study was to confirm previous study results about the inhibition of Ledermix on external root resorption, and to evaluate if the drugs contained in Ledermix may result in similar anti-resorptive properties when used individually after delayed replantation of teeth.

Materials and methods

Sixty-nine mature premolar roots in four beagle adult dogs were used in the study. The use of a dog model in this study was approved by Institutional Animal Care

and Use Committee (IACUC) of University of North Carolina at Chapel Hill. Ledermix paste was provided by Lederle Pharmaceuticals from Germany for use on dog teeth only in this study. 1% Triamcinolone cream and 3% Demeclocycline gel were made by Central Pharmacy in Durham, NC, USA. For all clinical procedures, general anesthesia was achieved with an IV injection of 6% sodium pentobarbital (30 mg kg^{-1} body weight).

The two-rooted premolars were first hemisected and then the roots were extracted as atraumatically as possible. The 69 roots were accessed and instrumented in an aseptic fashion. Instrumentation was performed with rotary nickel titanium files to the apical delta, with 1% NaOCl and 17% EDTA used for irrigation. Canals were dried with paper points, and then immediately filled as follows:

- Group 1 (negative control) was immediately replanted after root filling with GP and sealer (AH plus).
- Group 2 (positive control) was root canal filled with GP and sealer (AH plus), replanted after 60 min dry time.
- Group 3 (experimental group) was filled with Ledermix and replanted after 60 min dry time.
- Group 4 (experimental group) was filled with 1% Triamcinolone and replanted after 60 min dry time.
- Group 5 (experimental group) was filled with 3% Demeclocycline and replanted after 60 min dry time.

All teeth were sealed with Fuji IX glass ionomer before replantation. Because of the stability of the replanted teeth, splinting of the teeth was determined to be unnecessary. The dogs were maintained on regular diet after surgery, and then sacrificed after 4 months by an overdose of 6% solution of sodium pentobarbital administered intravenously. Block specimens containing the teeth and surrounding alveolar bone were prepared and fixed in 10% formalin. The tissue blocks were sectioned perpendicular to the long axis of the roots at a thickness of 5 μm at 90 μm, stained with hematoxylin and eosin, and examined with light microscopy.

Histological evaluation was conducted by two blinded investigators. Four sections per tooth were analyzed for the extent of root resorption and the type of interface between the root and surrounding tissue. For each section, an eight-point grid was superimposed on the section (Fig. 2) and at each of the eight circumferential points, the interface was judged as either Favorable Healing, or Unfavorable Healing.

Favorable Healing was identified by the presence of cementum over the dentin surface whether previously resorbed or not (Fig. 1). Unfavorable Healing was determined by the presence of inflammatory resorption or osseous replacement (Fig. 1). In addition, to evaluate the extent of root structure loss because of resorption, the original circumference of the root was determined. The radius of the remaining root structure was ranked on a linear, integer 0–6 scale at each of the eight points of the superimposed grid, with the value of six given to an unaffected radius and the value of zero given to a point without any remaining root structure (Fig. 2).

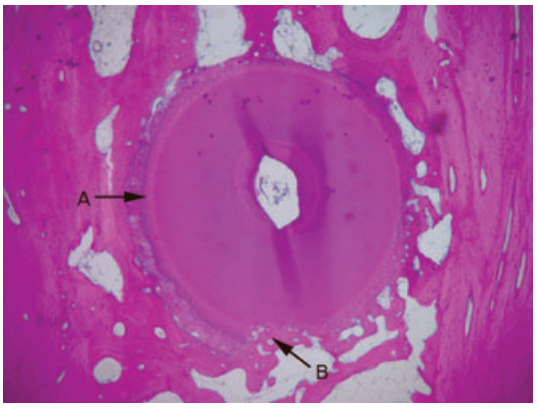

Fig. 1. Cross-section of root showing the healing patterns. Point a showing intact cementum and periodontal ligament was judged as Favorable Healing; Point b showing osseous replacement was judged as Unfavorable Healing.

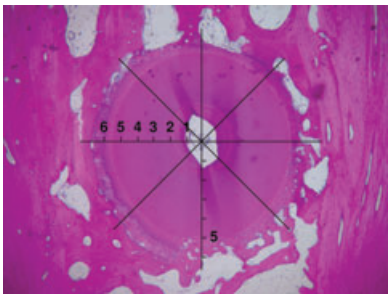

Fig. 2. Eight-point grid superimposed on histologic section. The radius of the remaining root surface was ranked on a 0–6 scale at each of the eight points of the superimposed grid, with the value of six given to an unaffected root surface, and the value of zero given to a point without any remaining root structure.

Data analysis

The chi-square statistics was used to compare the mean response for favorable healing. One way ANOVA was used to evaluate the remaining root structure. SAS Release 8.2 (Cary, NC, USA) was used for the statistic analysis.

Results

The healing patterns of the roots: favorable vs unfavorable

As shown in Table 1, the groups treated with Ledermix, Triamcinolone and Tetracycline had statistically significantly more favorable healing than the group filled with Gutta–Percha replanted after 60 min dry time (positive control) ($P < 0.05$). There was no statistically significant difference between Ledermix group and Triamcinolone group ($P > 0.05$), while the Tetracycline group showed less favorable healing than the negative control, Ledermix group and Triamcinolone group ($P > 0.05$).

Table 2 shows the percentage of the favorable healing points for all five groups in each of the four dogs evaluated. In every dog, the roots treated with Ledermix, Triamcinolone, and Tetracycline showed significantly more favorable healing than the roots filled with Gutta–Percha replanted after 60 min (positive control).

Table 1. Percentage of evaluation points with favorable healing by treatment method

	Group 1	Group 2	Group 3	Group 4	Group 5
	Negative control	Positive control	Ledermix	Triamcinolone	Tetracycline
Favorable healing	63.5	0	75.8	69.8	52.4
Unfavorable healing					
Inflammatory resorption	7.3	84.1	5.7	6.8	19.1
Osseous replacement	29.2	15.9	18.5	23.4	28.5

Table 2. Percentage of evaluation points with favorable healing by dog number

	Group 1	Group 2	Group 3	Group 4	Group 5
	Negative control	Positive control	Ledermix	Triamcinolone	Tetracycline
Dog 1	49.0	0	89.1	58.9	35.0
Dog 2	56.3	0	81.3	86.5	34.4
Dog 3	65.6	0	60.2	79.1	64.1
Dog 4	74.2	0	82.0	63.3	89.9

Table 3. Average remaining root structure (0–6 scale) of each group

	Group 1	Group 2	Group 3	Group 4	Group 5
	Negative control	Positive control	Ledermix	Triamcinolone	Tetracycline
Average radius of root	5.60	1.15	5.59	5.48	5.09

Table 4. Average remaining root structure (0–6) scale by dog number

	Group 1	Group 2	Group 3	Group 4	Group 5
	Negative control	Positive control	Ledermix	Triamcinolone	Tetracycline
Dog 1	5.58	1.16	5.84	5.48	5.19
Dog 2	5.48	2.71	5.72	5.59	4.31
Dog 3	5.55	2.47	5.24	5.52	5.20
Dog 4	5.68	0	5.76	5.37	5.63

The extent of root resorption

As shown in Table 3, the groups treated with Ledermix, Triamcinolone, and Tetracycline had statistically significantly more remaining root structure than the group filled with Gutta–Percha after 60 min dry time (positive control) ($P < 0.05$). There was no statistically significant difference between Ledermix group and Triamcinolone group ($P > 0.05$), while Tetracycline group showed less remaining root structure than the negative control, Ledermix group and Triamcinolone group ($P > 0.05$).

Table 4 shows the average remaining root structure for all five groups in each of the four dogs evaluated. In every dog, the roots treated with Ledermix, Triamcinolone, and Tetracycline had significantly more remaining root structure than the roots filled with Gutta–Percha after 60 min dry time (positive control) ($P < 0.05$).

Discussion

This study is one in a series of experiments using the canine trauma model that has investigated a variety of therapeutic and chemotherapeutic treatment modalities. Our protocol is consistent with the ones used in previous studies in order to make comparisons between studies. The methods we used for histological evaluation was presented by Andreasen (12), and has been used to evaluate the root healing by several other studies.

The trauma sustained by the root during the extra-oral dry time, whether because of desiccation, bacterial contamination or mechanical injury, determines the degree of PDL damage. The root surface, coated with dead PDL cells, serves as a potent inflammatory stimulus following replantation, and causes a more destructive inflammatory response including root resorption. We believe and the results of this and previous studies support that this secondary injury caused by inflammation is more severe than the initial injury itself. The results of this study demonstrated that corticosteroid and tetracycline, as anti-inflammatory and anti-resorptive agents, shut down or minimized the inflammatory reaction including clastic-cells mediated resorption, thus promoted more favorable healing than the positive control group which had no intracanal medicaments. Our study results have also confirmed the previous study results of the inhibition of Ledermix on external root resorption. There was no statistically significant difference between corticosteroid group and Ledermix group in either the number of favorable healing or the remaining root mass. We therefore feel confident that in severe traumatic injuries, where a large surface area of periodontal inflammation is expected, removing the pulp and placing corticosteroids into the canal at the emergency visit will become a standard protocol. Corticosteroid is more clinically useful than Ledermix as an intracanal anti-inflammatory medicament because it does not have the potential to stain the tooth.

The tetracycline group showed less favorable healing and remaining root structure than the negative control, Ledermix group and Triamcinolone group. A possible explanation is that tetracycline has the ability to bind to the tooth structure, especially the dentin. This binding might slow down its release to root surface through the dentinal tubules.

Similar previous studies have indicated that immediate replantation without any extra oral dry time (negative control) is likely to yield favorable healed surface in over 90% of the surfaces evaluated (1, 4, 13, 14). We obtained 63.5% favorable healing in our negative control group. In our study, we performed the endodontic treatment extra-orally in our negative control groups and tried to do it in the allotted 5 min. We felt it was important to perform the endodontic treatment extra-orally because all other groups were treated in this way. In hindsight we may

have sacrificed the quality of the root treatment or physically damaged the root surface when performing the procedure in such a rushed manner. Probably it would have been better to replant the teeth immediately and then come back a week later to perform the root treatment as recommended clinically.

The results of this study indicate that certain number of the roots in each group showed inflammatory root resorption. The root canal treatment was performed on vital teeth in an aseptic manner thus limiting canal infection as a stimulus for inflammatory root resorption. In addition the 60 min dry time is the model for producing osseous replacement rather than inflammatory root resorption. Thus it was surprising to find inflammatory root resorption. One possibility is that the root fillings were contaminated in the 4 months after root treatment. The dogs tended to wear away the coronal restorations, which could have leaked thus exposing the root filling to coronal leakage. Also the dogs are extremely susceptible to periodontal disease and bacteria may have penetrated through the sulcular area (15–17). Another possibility is that as osseous replacement is a dynamic process comprising a resorptive phase before the apposition phase we may see some inflammatory resorption in the specimens that are precursors to osseous replacement. As the incidence of inflammatory resorption was in proportion to that of the osseous replacement for all groups, the conclusions of the study should hold even if the inflammatory resorption later becomes osseous replacement.

When a tooth is separated from the socket, the periodontal ligament is ruptured. Majority of periodontal ligament cells were left on the root surface while some of them were left in the socket. After 60 min extra oral dry time, the survival rate of the PDL cells on the root surface is almost zero (1). Therefore, osseous replacement is predicted. However, in our study, the roots treated with Ledermix, corticosteroid and tetracycline demonstrated 76%, 70%, and 52% favorable healing, respectively. Even if these anti-inflammatory medicaments are able to shut down or minimize the initial inflammatory response, therefore inhibiting clastic-cell activity, there is a need for repairing cells such as osteoblasts to repopulate on the root surface in order to have cemental healing. In this experimental model, these repairing cells could only have come from the socket. A previous study has addressed the important role that the socket plays in the healing of replanted teeth. Teeth experiencing identical extra-oral dry time periods demonstrated significantly less healing as the age of the socket increased (14). Therefore, the environment provided by the socket appears to play a role in influencing the healing type of replanted teeth. Another possibility is that at 60 min not all the periodontal cells attached to the root surface have in fact died and it is the inflammatory response after replantation that destroys those cells remaining that have the potential for repair. Thus when the inflammatory response is shut down by the medication those cells with the potential for repair can proliferate and repair most of the root surface. These theories will have to be tested in experiments designed to test these hypotheses.

Triamcinolone is a weak corticosteroid. The concentration of triamcinolone we used in this study is 1%, which is very low. Previous study showed intracanal placement of corticosteroids was unlikely to result in any systemic side-effect even with the highest possible amount that could be used (18). Therefore, the anti-resorptive effect of some stronger corticosteroids such as Dexamethasone and Prednisone, or Triamcinolone with higher concentration should be evaluated in future studies.

As corticosteroids are biocompatible, intracanal placement of corticosteroids can be used as standard treatment protocol at emergency visit for traumatic injuries, in which the root resorption is predicted. Corticosteroids can also be placed in the canal space to intercept external inflammatory root resorption caused by infected pulp as long as the source of infection has been cleared out.

Acknowledgements

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