Pulpal response to bleaching of teeth in dogs

N. Sue Seale, DDS, MSD  Carolyn F.G. Wilson, DDS, MSD

Abstract

Three different application times of hydrogen peroxide and heat were used to evaluate removal of tetracycline stains from vital teeth of dogs and to evaluate histologic changes in the pulp tissue. Fifteen-, 30-, and 45-min treatments were applied at 2-week intervals for 4 consecutive treatments. The animals were sacrificed at 13, 62, and 92 days following the final bleaching treatment. The pulp tissue was examined histologically for treatment responses. Pulpal changes ranging from flattening and disruption of the odontoblastic layer to total obliteration of the odontoblastic layer and internal resorption were found in 17 of 18 treated teeth. The severity of the response appeared to be related to the length of treatment. The pulp tissues showed evidence of repair for all 3 treatment times in 5 of 6 teeth at the 92-day observation period.

Tetracycline, when ingested during the tooth-forming years, long has been known to cause unsightly staining of the dentition.1-6 Despite increased awareness on the part of physicians and patients and published warnings by drug manufacturers, tooth discoloration associated with tetracycline therapy continues to be a problem. Successful color removal by bleaching the teeth has been reported.7-11 Bleaching techniques vary, but one of the most widely reported procedures involves the use of externally applied 35% hydrogen peroxide and heat for from 20 to 45 min.8-10 There are conflicting reports concerning pulpal response to bleaching with hydrogen peroxide and heat.12-14 Cohen applied 35% hydrogen peroxide and 54°C heat for 15-min per surface for 3 treatments for a total treatment time of 45 min.12 He found no histologic changes in pulp tissue of human premolars when compared to control teeth. Robertson and Melfi applied 35% hydrogen peroxide and 46-51°C heat for 5-min intervals, in 2 appointments spaced 5 days apart to the buccal surface of human premolars.13 Histologic evaluation disclosed mild inflammatory responses limited to the superficial pulpal tissues of the experimental teeth which were significant when compared with the control teeth. Seale et al., evaluated the 2 vital bleaching components, heat and hydrogen peroxide, for their histologic effects on the pulp tissue of dog teeth.14 They applied 35% hydrogen peroxide and heat of 62°C separately and in combination for 30 min per surface for 4 consecutive weekly treatments. They found that 35% hydrogen peroxide alone produced severe but apparently reversible destructive changes in pulp tissue. The addition of 62°C heat did not appear to intensify the damage. Heat alone had no deleterious pulpal effect. None of the investigations involved tetracycline-stained teeth or measured color removal. The current study was undertaken as a part of a joint investigation to determine the most effective bleaching procedure with the least pulpal damage. The study also measured color removal as it related to application time of the bleaching agents.

Methods and Materials

Six healthy, approximately 14-month-old litter-mate mongrel dogs with newly erupted permanent dentitions were used as experimental animals. Five of the dogs were given feed mixed with tetracycline at a dose of 22 mg/kg for 90 days beginning at 6 weeks of age. The sixth animal received no drug and served as the control. The 5 experimental animals receiving tetracycline developed bright yellow-stained dentition (the control animal had white teeth). Four fully erupted, intact, noncarious canine teeth from each dog were the experimental teeth, whereas all others served as unbleached stained controls. The animals were anesthetized with 33 mg/kg pentobarbital IV prior to the bleaching procedures.
A rubber dam was used to isolate all 4 canine teeth simultaneously in each animal in order to protect the gingival tissue from hydrogen peroxide. The teeth were treated in the following manner:

**Tooth 1** — Cotton pledgets, 1.5 mm thick, saturated with 35% hydrogen peroxide, were applied to the labial aspect of the tooth from incisal tip to gingival border. The cotton pledget remained in place for 15 min and was heated with a constant temperature handheld device with a shaped metal tip. This tip was 17 x 8 mm and covered most of the cotton-covered tooth surface. It was heated to 62°C (144°F) and was applied gently to the entire cotton surface for 1 sec and then removed for 8 sec in a continuous routine for the 15-min treatment. The temperature of the metal tip was determined using a thermistor probe which had been calibrated to a temperature range of 140-147°F. Each tooth receiving heat treatment was in contact with the heated tip a total of 100 sec during the 15-min treatment period, while the cotton pledget was in contact the total time. The pledget was kept moistened with hydrogen peroxide by periodically touching it with another supersaturated cotton pledget. A total of 6 teeth, or 1 tooth from each animal, received this treatment, 2 observed for 13 days, 2 observed for 62 days, and 2 observed for 92 days.

**Tooth 2** — Cotton pledgets were applied and heated in the same manner as described for Tooth 1 and remained in place 30 min. A total of 6 teeth, or one tooth from each animal, received this treatment, 2 observed for 13 days, 2 observed for 62 days, and 2 observed for 92 days.

**Tooth 3** — Cotton pledgets were applied and heated in the same manner as described for Tooth 1 and remained in place for 45 min. A total of 6 teeth, or one tooth from each animal, received this treatment, 2 observed for 13, 2 observed for 62, and 2 observed for 92 days.

**Tooth 4** — This tooth received no treatment other than isolation by the rubber dam for 45 min. A total of 6 teeth received this treatment and served as the control teeth, 2 each for the 13-, 62-, and 92-day observation periods. The total number of teeth involved was 24 (18 experimental, 6 control).

The 6 animals were treated 2 weeks apart for 4 consecutive treatments. The animals were anesthetized with pentobarbital and sacrificed with potassium chloride for removal of the 4 experimental teeth at 3 observation periods following the last hydrogen peroxide treatment: 13 days, 62 days, and 92 days. Two animals were sacrificed at each observation period. Each animal received bilateral perfusion of the carotid arteries with 200 ml normal saline followed by 10% buffered formalin, until the jugular catheters ran clear.

The teeth were extracted following perfusion of the head. The root tips were severed to allow for better infiltration of fixative. The teeth were fixed in 10% buffered formalin for 1 month. Following fixation, the teeth were demineralized and embedded in Paraplast Plus. Each tooth was sectioned at a thickness of 5 μ and every tenth section was collected. Twenty samples from each tooth were collected and stained with hematoxylin-eosin and examined. The pulp was examined and evaluated for its response in the coronal, middle, and apical thirds. Results were recorded for each area according to the following criteria: (1) amount and quality of reparative dentin, (2) presence and intensity of calciotraumatic lines in dentin, (3) integrity of odontoblastic layer and predentin, (4) morphology of odontoblastic cells, (5) evidence of internal resorption, (6) presence and type of inflammatory cells, (7) degree of inflammatory response, and (8) evidence of hemorrhage and edema. Differences in type and severity of response between treatment times and between observation periods were noted and compared.

**Results**

Two teeth for each treatment procedure, or a total of 8 teeth, were available at each observation period. The distribution of teeth according to observation time is shown in Table 1.

**13 Days Following Final Treatment**

The 2 teeth receiving the 15-min application of hydrogen peroxide and heat showed different reactions at this early time period. One tooth demonstrated a marked response in the incisal tip with a narrow zone of amorphous reparative dentin, absence of predentin, flattened and discontinuous odontoblasts, and eosinophilic staining with vacuoles throughout the pulp tissue (Fig 1). The odontoblasts returned to normal, with regular tubular dentin and predentin in the middle and apical pulp. The other tooth showed no

**TABLE 1. Distribution of Experimental Teeth According to Observation Times (n = 24).**

<table>
<thead>
<tr>
<th>Group</th>
<th>13 Days</th>
<th>62 Days</th>
<th>92 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-min treatment</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>30-min treatment</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>45-min treatment</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>No treatment control</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total teeth in each observation time</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>
response and was indistinguishable from the control tooth (Fig 2).

The teeth in the 30-min treatment sample showed responses of 2 different degrees of severity in the incisal pulp tissue, but were comparable in the middle and apical thirds. The tooth demonstrating the more severe reaction revealed a dense infiltrate of polymorphonuclear leukocytes, atubular reparative dentin with trapped cell bodies and flattened, discontinuous odontoblasts. The other tooth demonstrated a less severe reaction with atubular reparative dentin, absence of predentin, and flattened odontoblastic cells which were absent in places (Fig 3).

The teeth treated for 45 min exhibited results similar in severity to those in the 30-min group, including the 2 different degrees of severity in the incisal pulp tissue. The tooth demonstrating the more severe response revealed a dense inflammatory infiltrate, atubular reparative dentin and flattened odontoblasts. The tooth demonstrating the less severe reaction showed some inflammatory cell infiltrate, amorphous reparative dentin, and flattened and discontinuous odontoblasts (Fig 4).

62 Days Following Final Treatment

The two teeth receiving the 15-min application of hydrogen peroxide and heat showed pulpal re-
responses at the incisal tip of atubular reparative dentin, shortened odontoblasts, and a mild chronic inflammatory infiltrate (Fig 5). Odontoblasts and dentin in the apical region assumed a normal appearance.

The two 30-min treated teeth showed pulpal response of the incisal tips involving thick amorphous reparative dentin with cell bodies trapped and shortened and occasionally missing odontoblasts (Fig 6). There was a mild chronic infiltrate which lessened as the apex was approached. The odontoblasts returned to normal with calcitroumatic lines appearing in the tubular reparative dentin and disappearing in the apical area.

The two 45-min treated teeth varied in the severity of their response with 1 showing more active destructive changes and the other showing healing of apparent earlier destructive changes. The incisal tip of 1 tooth revealed no reparative dentin or odontoblasts. There were large numbers of giant cells involved in active and extensive internal resorption (Fig 7). Moving down from the incisal tip the pulp tissue appeared as granulation tissue with a moderate mixed inflammatory infiltrate and hemorrhage. There was a gradual reappearance of flattened odontoblasts which were involved in repair of previous areas of internal resorption. Apically, the odontoblasts became more normal with predentin, tubular reparative dentin, and calcitroumatic lines, but the hemorrhage and inflammatory cells persisted. The second tooth appeared to be in a later stage of response as there were numerous areas of repaired internal resorption with cellular inclusions and a continuous flattened odontoblastic layer. The pulp was infiltrated with chronic inflammatory cells. In the middle of the pulp and apically, the structures all returned to a more normal appearance with 4 calcitroumatic lines clearly visible.

92 Days Following Final Treatment
The teeth receiving the 15-min treatment showed a large amount of amorphous reparative dentin in the incisal tip (Fig 8). The odontoblasts in this region were shortened in height and abnormal in shape but continuous. The pulp tissue stained eosinophilically. The odontoblasts along the middle third assumed a more normal shape with reappearance of predentin and tubular reparative dentin. Odontoblasts of the apical third were of normal height and shape. The pulp tissue appeared similar to that of the control teeth. The 30-min treated teeth showed shortened abnor-
FIG 8. Ninety-two days following the final 15-min treatment, the pulp in the incisal third revealed eosinophilic staining, shortened odontoblasts, and thick amorphous reparative dentin (90x).

FIG 9. Ninety-two days following the final 45-min treatment 1 tooth revealed large amounts of amorphous reparative dentin, cellular inclusions, and numerous areas of repaired internal resorption in the incisal third (90x).

Discussion

There appears to be a wide variety of responses represented in this investigation. The differences in severity of pulpal reaction across treatment times at the same observation period appears to be related to the length of heat and peroxide application time with the longer application times showing progressively more severe pulp pathology.

The results of this investigation would appear to substantiate an earlier report14 of severe but apparently reversible destructive changes in the pulps of dog teeth in contact with 35% hydrogen peroxide alone and in combination with 62°C heat for four 30-min applications. They found similar pulpal responses of internal resorption and inflammation and speculated that the porous nature of the enamel and dentin allowed the agent to penetrate the tooth surface sufficiently to affect the odontoblasts and pulp tissue.

These current findings of increasingly severe pulpal responses as bleaching agent application times increase would be consistent with enamel and dentinal penetration by the agents. The longer the solution is in contact with the enamel surface, the deeper the penetration and the greater the quantity of solution to penetrate. Cohen,12 and Robertson and Melfi13 reported mild or no pulpal changes associated with vital bleaching techniques. However, they were not examining times of application which have been reported to be clinically successful for color removal. A second reason for the differences in pulpal response in their studies compared with the present investigation may be related to the particular teeth present throughout the entire pulp, but there were no signs of hemorrhage or edema. All structures appeared normal in the apical third.
involved. Both Cohen and Robertson and Melfi used human premolar teeth. The average thickness of enamel and dentin was not given; therefore, a direct comparison cannot be made. However, El-Hadary et al.\textsuperscript{16} reported an average thickness of 3.8 mm for enamel plus dentin in human premolars. The average thickness of the enamel and dentin in the dog teeth in the present study was 1.7 mm, thus making a difference in enamel and dentin thickness of 2.1 mm. If the teeth in the Cohen and Robertson and Melfi studies were similar in thickness to those reported by El-Hadary, then the thinner structure of the dog teeth may have allowed the bleaching solution to penetrate to the pulp more readily. The bleaching procedure under study is performed most often on human maxillary central and lateral incisors. The average thickness of enamel and dentin in human incisors is more similar to that of dog canine teeth than that found in human premolars. If the thickness of enamel and dentin is a critical factor in permitting hydrogen peroxide to affect the pulp adversely, this study more accurately demonstrates the probable effect of bleaching on the pulp of human maxillary anterior teeth than does the study which examined this phenomenon in human premolars.

All but one of the teeth in the present study showed resolution and healing of earlier changes, which would appear to indicate that the damage caused by all 3 treatment times may be of a reversible nature. There was 1 nonvital tooth in the 92-day observation period for the 45-min treatment which represented 50% of the teeth in that group. This finding would most certainly contraindicate the 45-min treatment as it was done in this study. However, the associated clinical study of color removal indicated that only two 45-min treatments are necessary for successful color removal while this study reports pulpal changes associated with 4 treatment sessions.

The short observation times and the small numbers of teeth examined preclude direct clinical application of the results of this study. However, the consistent findings of pulpal responses in 17 of 18 teeth receiving hydrogen peroxide and heat would indicate the need for further studies involving longer observation periods and including greater numbers of teeth.

Conclusions

1. Heat of 62°C combined with 35% hydrogen peroxide applied to the external surface of tetracycline-stained dog teeth for 4 sessions each of 15, 30, or 45 min, produced observable pulpal changes in 17 of 18 teeth treated.

2. The severity of the pulpal response appeared to be associated with the length of treatment time (the longest time causing the most severe changes).

3. The pulp tissues showed evidence of repair for all 3 treatment times in 5 of 6 treated teeth in the 92-day sample.

Dr. Seale is an associate professor and Dr. Wilson is an assistant professor, pedodontics, Baylor College of Dentistry, 3302 Gaston Ave., Dallas, TX 75246. Reprint requests should be sent to Dr. Seale.