Enriched collagen solution as a pulp dressing in pulpotomized teeth in monkeys

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Abstract

The purpose of this study was to assess the pulp healing process in baboon teeth after pulpotomy using an enriched collagen solution (ECS) as a pulp dressing. Twenty-five noncarious permanent teeth of two young baboon monkeys were pulpotomized under a rubber dam. After coronal pulp resection and hemostasis, ECS was applied on the pulp stumps and covered with sterile dental wax. The controls were covered with wax only. The cavities were sealed with IRM. The ECS was prepared from native, acid-soluble monkey skin collagen. The concentrations used were 0.25-0.3% solution in neutral 0.4M NaCl buffered with 0.005M tris. Two months after treatment the animals were sacrificed by perfusion with 10% formalin solution and the teeth prepared for histologic examination. The results indicated that 80% of the treated teeth had vital pulps, as compared to 20% of control teeth. In the ECS-treated pulps, dentin bridges were present in 73% of the teeth vs. 30% in the controls. In many of the ECS-treated pulps, cells were seen proliferating through the incomplete dentin bridge into the pulp chamber. More than half of the ECS-treated teeth showed no pulpal inflammation after two months.

Although preventive measures have reduced the incidence of dental caries,1 pulp involvement still remains a common clinical problem. In permanent teeth, pulp exposures usually are treated by conventional endodontic procedures. The formocresol pulpotomy is the treatment of choice for pulp exposure in vital primary teeth.2-7 However, despite the widespread use of this procedure, its usefulness had been questioned; histological examination of dental pulps in dogs,8 monkeys,8 and humans10 revealed inflammation, internal resorption, fibrosis, and necrosis of the residual radicular pulp following formocresol treatment. Moreover, the mutagenic and carcinogenic potential of formocresol has been demonstrated.11 Thus, the need for a more biologically acceptable pulp dressing following pulpotomy in primary teeth became evident. Several investigators have tested glutaraldehyde and a diluted (4%) solution of formocresol. Although both materials appeared to be less deleterious to pulp tissue than the original Buckley's formula, neither resulted in a normal histologic appearance of the residual pulp tissue.12,13 Recently, complete pulpal healing has been achieved in the dog 30 days after pulpotomy using an enriched collagen solution (ECS) as a pulp dressing.14

This article reports results obtained from further examination of the efficacy of ECS as a dressing agent using the baboon monkey as a test animal for a protracted period of time (two months).

Methods and Materials

The native ECS preparation was made as previously described.15 Acid-soluble native collagen was extracted with cold 0.5M acetic acid from the skin of a young monkey and further purified using the trichloroaceticethanol method, lyophilized, and kept at -20°C in vacuo until used. Before use, a 0.4% collagen solution in 0.4M NaCl-0.01M tris, pH 7.6 was prepared, to which an equal volume of Medium 199 was added. All procedures were performed under sterile conditions.

The experiments were carried out in 29 noncarious permanent teeth of two young baboon monkeys. Preoperative radiographs were taken to assess the state of root development and the absence of pulpal or periapical pathology. All the teeth had complete roots and closed apices. Treatments were done under ster-
TABLE. The Effect of ECS on Pulpotomized Teeth: Histologic Findings

<table>
<thead>
<tr>
<th>Number of Teeth Treated</th>
<th>Experimental (ECS + Wax)</th>
<th>Controls (Wax only)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Vitality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No necrosis</td>
<td>12 (80%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Partial necrosis</td>
<td>2 (13%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Total necrosis</td>
<td>1 (7%)</td>
<td>6 (60%)</td>
</tr>
<tr>
<td>Absent or mild</td>
<td>8 (53%)</td>
<td>—</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>4 (27%)</td>
<td>2 (20%)*</td>
</tr>
<tr>
<td>Severe</td>
<td>3 (20%)</td>
<td>2 (20%)*</td>
</tr>
<tr>
<td>Regular</td>
<td>7 (47%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Irregular</td>
<td>6 (40%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Odontoblastic layer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>2 (13%)</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>Dentin bridge</td>
<td>11 (73%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Reparative dentin</td>
<td>12 (80%)</td>
<td>4 (40%)</td>
</tr>
<tr>
<td>Calcifications</td>
<td>5 (33%)</td>
<td>1 (10%)</td>
</tr>
</tbody>
</table>

* Remaining vital teeth.

Figure 1. Distal root of a pulpotomized mandibular first molar treated with ECS. Notice the apparently complete dentin bridge (db), atubular dentin formation (ad) with cells entrapped at the entrance of the canal, and connective tissue proliferation (ct) coronal to the bridge [80 x - hematoxylin and eosin (H & E)].

Figure 2. Another section of the same tooth as in Figure 1. Interruption in the continuity of the bridge (db) can be observed (arrow). Regular tubular dentin (td) is evident at the canal wall. Connective tissue proliferation (ct) is present coronal to the bridge [80 x (H & E)].

The cavity preparations of treated teeth were sealed with reinforced zinc-oxide eugenol cement IRM.* Postoperative radiographs of all teeth were taken one and two months after treatment. The animals then were sacrificed by perfusion with 10% formalin solution. The jaws were dissected immediately and the base part cut off. This exposed the apices of the teeth facilitating fixation of the pulp tissue. The remaining part of the jaw, with the teeth in position, was im-

1. Fifteen teeth were treated with ECS, and covered with sterile dental wax.
2. Ten teeth were treated with sterile dental wax (DW) only.
3. Four teeth were left intact.

* IRM — The L.D. Caulk Co.; Milford, DE.
mersed in 10% buffered formalin and demineralized in 10% EDTA. After demineralization, the teeth were trimmed, embedded in paraplast, and cut longitudinally to obtain serial 6 μm sections. The sections were stained with hematoxylin and eosin, and examined under a light microscope. The results were assessed by “blind” testing of the different microscopic preparations, and ranged according to a modification of the criteria by Horsted et al.\(^{16}\) as follows:

1. State of pulp vitality — Presence and extent of necrosis
   a. No necrosis
   b. Partial necrosis — Areas of necrosis at the wound surface or in part of the root pulp
   c. Total necrosis
2. Presence and extent of inflammation
   a. Absent or Mild — Normal pulp or a few inflammatory cells limited to the bridge area
   b. Moderate — Inflammation evident below the bridge but limited to the coronal third of the radicular pulp
   c. Severe — Inflammatory cells and circulatory disturbances affecting most of the pulp
3. Presence of a dentin bridge
4. Presence of reparative dentin along the canal, below the dentin bridge area
5. Presence and regularity of an odontoblastic layer
   a. Regular — Present all along the root canal
   b. Irregular — Interrupted or existing in only part of the pulp canal
   c. Absent — No odontoblastic layer evident
6. Presence of calcifications in the pulp, not related to the bridge.

**Results**

All the teeth presented normal radiographic appearance, with no signs of pulpal or periapical pathosis. Dentin bridges were evident in the anterior teeth of the ECS group.

The treatment results from the ECS-treated pulps were consistently more favorable than those of the control teeth and are incorporated in the Table. Twelve of 15 teeth in the ECS group (80%) had vital pulps after 60 days, while only 20% of the controls showed no signs of necrosis. Dentin bridges were present in 73% of the experimental group and in only 30% of the control teeth. Examination of the serial sections revealed solid and continuous calcified bridges with entrapped cells in some sections, while perforations of the bridge were seen in others. (Figures 1 & 2). An unusual finding was the presence of both connective tissue cells and blood vessels coronal to the incomplete bridge in more than half of the ECS-treated pulps (Figure 3). No such findings could be observed in any of the control teeth.

Reparative dentin was present in 80% of the ECS group and in 40% of the controls. This dentin was tubular in the bridge and tubular dentin was formed along the canal walls (Figure 4a). Either no inflammation, or very few inflammatory cells over the bridge area in its immediate vicinity were seen in 53% of the ECS-treated teeth (Figure 5), and only 20% of the treated teeth showed severe inflammation extending deep into the pulp. In the controls, 60% of the pulps were totally necrotic and the rest of the teeth showed moderate to severe inflammation (Figures 4a-c). Only one of the teeth of the ECS group exhibited a large, well-defined, calcified mass located in the middle of the pulp canal. In two others there were small calcified bodies, and in the remaining two teeth of the ECS group and in one tooth of the DW group, diffuse calcifications were found along the root canal.

**Discussion**

The beneficial effect of exogenous ECS on healing processes of a variety of wounds including burns and pulp tissue has been recognized for some time.\(^{14,15,17,18}\) In this study, however, a new, striking, and hitherto undescribed phenomenon has been observed — namely the proliferation of connective tissue cells located coronally to the newly formed dentin bridge. Thus, the results obtained from this study differ from those of an earlier study by Nevins et al.\(^{19,20}\) They used a collagen-calcium phosphate gel paste and observed dentin formation peripherally to existing pulp and at the tissue-paste interface, but no tissue infiltration of the space occupied by the paste. How can the presence of the cells be explained? The possibility
FIGURES 4. a-c. Maxillary central incisor of the experimental group (ECS) rated with moderate inflammation. a. (left) Dentin bridge area — A small, incomplete dentin bridge (db) containing remnants of the collagen material (cm) is evident. Also note a regular odontoblastic layer (ol), regular tubular dentin (td), and predentin (p). Inflammatory cells (ic) are present below the bridge \[125 \times (H \& E)\]. b. (center) Coronal third of radicular pulp — The inflammatory infiltrate (ic) is limited to this area. Note the regular odontoblastic layer (ol) and predentin (p) \[125 \times (H \& E)\]. c. (right) Middle third of the radicular pulp presenting an apparently normal pulp (np) \[125 \times (H \& E)\].

FIGURE 5. Pulpotomized maxillary lateral incisor treated with ECS. Note remnants of the collagen material (cm) and reparative dentin (rd). Inflammatory cells (ic) are present over the bridge and in its immediate vicinity — mild inflammation \[80 \times (H \& E)\].

exists that the cells coronal to the dentin bridge were there before the bridge was formed and that its formation resulted from the cellular activity of the same cells, starting at the interface between the sound pulp odontoblasts and the collagenous dressing. It also may be theorized that the dentin bridge formation and cellular proliferation coronal to the formed bridge took place as two independent processes at essentially the same time. To answer this rather important question, one has to investigate the dynamics of the process earlier than two months and at several shorter time intervals.

Another immediate question posed by the appearance of connective tissue structures coronal to the dentin bridges is that of the ultimate fate of these cells. The fact that one can see both cement-like structures and odontoblast-like cells — as well as vascularity — indicates the viability of these structures and possible metabolic activity. This justifies the belief that the whole pulp chamber ultimately would reconstitute and partly resume its normal function. To verify this hypothesis, long-term experiments must be conducted. In this study it appears that native collagen preparations used as pulp dressing agents seem to be superior to any other hitherto used methods, including the widely used calcium hydroxide. The latter has been reported to bring about the formation of calcified dentin bridges. Induction of hard tissue formation, however, results from mild irritation of coagulation necrosis caused by calcium hydroxide. The coagulated tissue calcifies and dentin subsequently is formed by newly differentiated odontoblasts. An entirely different mechanism is observed in the ECS-induced viable tissue, in which true dentin-like tubular structures are formed. Moreover, dentin bridge formation per se is not a sign of healing since bridges have been formed even with formocresol in monkeys' teeth.

Conclusions

Although the results obtained from this and other studies using ECS as a pulp capping agent are promising, it should be noted that so far the experiments were carried out on noninflamed pulps, while clinical pulp exposure hardly ever occurs without an inflammatory reaction. The effect of ECS on inflamed pulp should continue to be studied before reaching definitive conclusions about its clinical efficacy.

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Quotable quote: children in peril

Developing countries are not a homogeneous entity. They are at various stages of socioeconomic development and are developing at various speeds. But they all face one most important problem — high infant and child mortality rates and morbidity. In India, for example, the infant mortality rate is around 129/1,000 live births; more than 50% of infant deaths occur within the first month of life; and low birth weights are found in almost a third of all births. For mothers younger than 20, the birth weights are significantly lower than for mothers from 20 to 24. The frequency of low birth weight increases with rising birth orders. The story of infant and child health in the Third World is one of needless illnesses, avoidable disabilities, and missed human opportunities. Acute diarrheal disease is the leading cause of death in children younger than one year of age. Malnutrition, overcrowding, lack of protected water supplies, poor environmental sanitation, and low levels of education all act together in a vicious cycle.

The Third World experience during the past two decades has shown that with able leadership, well-designed and effectively operated programs, appropriate technologies, and forms of health care delivery together with professional back-up support, infant mortality rates can be reduced by 50% or more even by poor countries in a relatively short period of time and at a cost less than the equivalent of 2% of annual per capita income.

Ramalingaswami V: Children in peril. The Unesco Courier, April, 1984