Selectively preventing development of third molars in rats using electrosurgical energy

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The third molar is a tooth of questionable value in 21st-century humans and one that causes pain, suffering and expense to millions of people worldwide every year. A clinical dilemma arises for dentists who are trying to manage the oral health of patients who develop third molars. If dentists recommend prophylactic extraction early in the patients’ lives before any signs or symptoms of disease are present, the patients face the physical and emotional trauma of surgical extraction, as well as the risks and morbidity associated with surgery. If dentists do not recommend prophylactic extraction, patients face an uncertain future, with the possibility of pain and disease from this tooth and the likelihood that if extraction becomes necessary, the surgical removal will occur with a greater morbidity. In spite of a 1979 National Institutes of Health, or NIH, conference that addressed the management of the third molar and the clinical dilemma that it poses to clinicians and their patients, evidence suggests clinicians have not reached a consensus of agreement on how best to manage this tooth.

Few novel therapeutic solutions have been proposed to address the problems caused by third molars. Enucleating uncalcified third molars in children aged 7 to 9 years old was proposed in 1936 by Henry and was practiced for a period by Ricketts and colleagues in an attempt to reduce the surgical morbidity from extraction. In this procedure, the third molar is enucleated in a surgical procedure before calcification of the crown is complete and when the developing tooth is located in its crypt just below the surface of bone. Although evidence suggests that there may be reduced morbidity compared with extraction at later
Since third molars have no other molars developing distal to them, successfully stopping third molars from forming cannot interfere with the development of any tooth posterior to them.
bud formation. Our study targeted only the maxillary third molar area since the maxillary tuberosity region is more readily accessible compared with the mandibular ramus region in the tiny rat pup mouth.

This study tested the hypothesis that third molars can be selectively prevented from developing in the rat animal model using electrosurgery at approximately the time when tooth bud initiation is occurring.

MATERIALS AND METHODS

Thirty-three edentulous neonate Sprague-Dawley rat pups received a single, unilateral, momentary pulse of monopolar electrosurgical energy to one of their maxillary tuberosity regions via a 30-gauge, stainless steel electrosurgical probe inserted into a custom-made, pre-fabricated, plastic intraoral positioning device. The contralateral sides of the pups' mouths received no treatment and were the control. Sixteen 3-day-old, six 5-day-old and 11 10-day-old rat pups received treatment. We fabricated the insulating plastic positioning devices that housed the electrosurgical probes before actual treatment using the mouths of euthanized rat pups of the same age as the rats that were to be treated. The cutting tip of the electrosurgical probe extended beyond the insulated plastic positioning device to target only the most distal aspect of the tuberosity area of the maxilla. The stainless steel tip of the electrosurgical probe extended less than 1 mm beyond the plastic positioning device to ensure contact with the oral mucosa of the maxillary tuberosity. Clipping the grounding wire of the electrosurgical unit to gauze saturated with conduction fluid and laying the rat pups on the gauze during treatment grounded the animals. The rat pups received inhalation anesthesia until they were motionless via isolation in a closed vessel for approximately one minute. We placed the prefabricated positioning device into the animals’ mouths and secured it in place by gently holding the animals’ jaws together. The activated cutting tip of an electrosurgery unit momentarily touched the 30-gauge stainless steel probe of the positioning device extending outside the pups’ mouths to deliver a 90-watt pulse of energy to the tuberosity region. After treatment, the pups recovered on a heating pad and reunited with their nursing mother rats.

The pups received continuous care from their mother rats until they were weaned at approximately 30 days of age. The rats lived in standard caged housing using a 12-hour–light/12-hour–dark cycle with food and water provided ad lib throughout the experimental period. The animals received carbon dioxide inhalation euthanasia when they were between 47 and 52 days old, which is an age several weeks older than the date at which third molars normally erupt. After the rats were sacrificed, we dissected them for gross intraoral and radiographic examinations. If a third molar was present, we compared its size with the third molar on the same animal’s contralateral untreated control side.

We chose two euthanized rats with vastly differing maxillary development to evaluate histologically. One rat had no third molar on its
treated side and a nearly normal tuberosity and maxillary development, while the other rat had no third molar and marked palatal asymmetry with accompanying facial deformity. We took tissue specimens of these rats from the tuberosity regions on their treated sides (in the area distal to the second molar in which no third molar development had occurred) and had these tissue specimens examined for evidence of third molar development and cyst formation. Additionally, we had these tissue samples of the treated sides compared histologically with a tissue sample taken from the tuberosity region distal to a third molar on a control side of one of the rats. Histologic sections were made through the frontal plane at 500-micrometer intervals, stained with hematoxylin and eosin, and evaluated by Pathology Associates, Advance, N.C.

RESULTS

Thirty of the 33 rat pups survived the experimental period. None died while under anesthesia, and all of the pups appeared healthy and to be nursing 24 hours after they returned to the mother rats. Three rats subsequently died for unknown reasons. Fourteen rats showed no apparent effects from the electrosurgery treatment (Figure 2). Sixteen rats demonstrated clinical and radiographic differences in third molar development on their treated sides compared with their untreated sides. Ten of these rats did not develop third molars on their treated sides (Figure 3), while the remaining six rats had noticeably smaller third molars on their treated sides (Figure 4, page 1402). The table shows the results of the clinical and radiographic examinations and the ages of the rats.
when they were treated.

The electrosurgical treatment also affected the rats’ palatal development. Only two rats missing third molars appeared to have nearly normal development of their palates (Figure 3). We observed two general types of palatal developmental changes in the other rats. One type affected only the local topography of the tuberosity region. The other affected its overall symmetry, especially noticeable in the area of the rugae. The two types of palatal deformities often occurred independently of one another; some animals had changes only in the area of the tuberosity, while other animals had changes that were only reflected by the asymmetry of the rugae. Some animals possessed both types of palatal deformity.

The histologic evaluation of the tissue specimen taken from the tuberosity region distal to the third molar on the untreated control side showed some local gingival inflammation, but otherwise it appeared normal (Figure 5, page 1403). The histologic evaluation of the tissue sample taken from the tuberosity region distal to the second molar of a treated side with a missing third molar and nearly normal maxillary development revealed no evidence of third molar tissues, cyst formation or abnormal tissue changes (Figure 6, page 1403). The histologic evaluation of the tissue sample taken from the tuberosity region distal to the second molar of the treated side of the animal that had severe maxillary growth disturbance, rugae asymmetry and facial deformity revealed no evidence of third molar tissues or cyst formation. It did, however, show other marked degenerative tissue changes (Figure 7, page 1404).

**DISCUSSION**

Selectively preventing third molar development without detrimentally affecting other developing
tissues such as the jawbone and nearby teeth is a challenging goal, especially in the tiny rat pup mouth where other critical developing tissues lie in close proximity to one another. The teratogenic agent must target selectively and specifically only the distally migrating dental lamina, the biomolecular events of the interaction between the dental lamina with highly specific jaw mesenchyme, or the earliest developing stages of tooth bud initiation. Otherwise, damage to other developing tissues will occur. When undesirable growth disturbances on the developing maxilla occurred, the electrosurgical energy likely had damaged developing bone. The wide range of developmental effects on the jaw most likely were due to the inability to control and limit the application of the electrosurgical energy specifically to the developing tissues of the third molar.

In a typical clinical setting, an electrosurgical unit is minimally invasive and highly controlled.

**TABLE**

<table>
<thead>
<tr>
<th>COMPARISON OF THE THIRD MOLAR ON THE TREATED SIDE WITH UNTREATED CONTROL SIDE</th>
<th>NO. OF RATS</th>
<th>AGE OF RATS AT TIME OF TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Equal in Size</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Smaller in Size</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Third Molar Completely Missing</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>30</strong></td>
<td><strong>15</strong></td>
</tr>
</tbody>
</table>

**Figure 4.** A. Intraoral view of adult rat with smaller maxillary third molar on treated right side (arrow S). Untreated left side shows a normal-sized maxillary third molar (arrow U). B. Radiograph of adult rat with smaller maxillary third molar on treated right side (arrow S). Untreated left side shows a normal-sized maxillary third molar (arrow U).
Its effects are derived by damaging tissues with heat. The energy concentrated at the cutting tip causes an explosive rapid temperature rise in the cells closest to the tip, and a slower, lower temperature rise as its current passes deeper through the tissues.25 The cellular effects from the electrosurgical energy range from pyrolysis and vaporization at temperatures of more than 100°C to cell wall damage, DNA and RNA denaturation, and enzyme deactivation at temperatures closer to 60°C. When the tissue being removed is large enough to be seen by the operator and when the tissue is at a safe distance from other vulnerable tissues, the effects of electrosurgery can be very local and precise.

However, when electrosurgical energy is applied near the invisible tooth anlage in the tiny mouth of newborn rats, the effects of the electrosurgical energy cannot be nearly as local or precise. The embryonic tooth-forming tissues of the third molar lie fractions of a millimeter below the oral mucosa26 and cannot be seen. As a result, it was not possible to predictably protect and isolate the vulnerable developing bone from the energy and heat of the electrosurgical energy. The result was a relatively large, unpredictable area of tissue damage during treatment and a wide range of bony developmental effects seen after the rats were euthanized.

In the 14 treated rats that developed third molars and showed no effects from the treatment, the electrosurgical energy may have missed its intended third molar target and any developing tissues. In the two rats that developed no third molars and showed no apparent clinical or radiographic signs of damage to the maxilla, the treatment appears to have affected the intended tooth-forming tissues without damaging its bony surroundings significantly. In the six animals with smaller-than-normal third molars, electrosurgical energy may have caused partial ablation of tooth-forming tissues or may have acted as a thermal teratogen, altering normal embryologic tooth development by slightly elevating their temperatures. Temperature increases of as little as 1.5 to 2.5°C may cause teratogenic effects on developing tissues.27

The maxillary tuberosity is an active site for growth for the maxilla.28 Minor damage to this site during treatment could explain the local topographical changes observed in that region. The rats that displayed asymmetry of their palates, especially that evident in the area of the rugae, likely sustained more extensive damage to the developing bone during treatment. Unilateral electrosurgical treatment of these animals appears to have altered the degree of displacement growth of the treated side compared with
the displacement growth of the untreated side.

Selectively prevented third molar development in the rats occurred with little to no observable collateral destructive tissue effects to the developing bone in several of the animals. The difficulty of targeting only the microscopic epithelial and mesenchymal anlage of the tooth-forming tissues without also detrimentally affecting surrounding developing tissues such as bone also was illustrated, as many of the animals that were missing third molars also possessed abnormal maxillary development to a greater or lesser extent. The key factor in selectively stopping third molar development is limiting teratogenic effects to only the developing tissues of the third molar, and the nonselective, invasive electrosurgical energy used in the study was not able to accomplish it predictably without frequent collateral damage.

For humans, a successful methodology to prevent third molar development near the time of tooth bud initiation must be not only highly selective but also minimally invasive or noninvasive, atraumatic and relatively easy to apply. That two test animals displayed a nearly ideal result—completely eliminating the third molar development without grossly disturbing the development of the maxilla—offers hope that selective agenesis might be achievable using a less invasive, more selective teratogen. However, the fact that only limited success occurred in some of the test animals—either a third molar merely reduced in size or a missing third molar accompanied by some degree of deformity of the maxilla—points out some of the difficulties that will be encountered in achieving a clinically acceptable therapeutic result in humans and some of the dangers associated with it.

CONCLUSIONS

A momentary pulse of electrosurgical energy can selectively stop the development of the third molars in neonate rat pups without cyst formation or other significant tissue changes. However, growth disturbances to the developing maxilla will occur if the energy is not confined to the anlage of the third molar or the developing tooth bud itself. In the rat animal model, electrosurgical energy is too powerful and indiscriminant in its tissue destruction to ablate tooth-forming tissues selectively without causing other unpredictable and undesirable collateral growth disturbances.

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