Wound Healing in Beagle Dogs after Palatal Repair without Denudation of Bone

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The connection of scar tissue to palatal bone by Sharpey's fibers, after cleft palate surgery, might lead to maxillary growth inhibition. The aim of this study, in beagle dogs, was to investigate the possibility of preventing the development of Sharpey's fibers by means of a modified surgical technique. In group 1, palatal repair according to von Langenbeck, was simulated. In group 2, palatal surgery was performed using a new partially split flap technique. The palates were histologically evaluated 12 weeks after surgery and compared with a control group. In group 1, the scar tissue was firmly attached to bundle bone by means of Sharpey's fibers. In group 2 and in the control group, this kind of attachment was not found; the bone was of the lamellar type. The partially split flap technique had led to the development of vaguely demarcated scar tissue and it had prevented, to a large extent, development of Sharpey's fibers.

KEY WORDS: cleft palate, dogs, surgery, wound healing

Forty years ago, Graber (1954) reported detrimental effects of cleft palate repair on facial growth in humans. Nowadays, surgery is considered one of the major factors causing disturbances in facial growth in cleft palate patients (Ross and Johnston, 1972; Ross, 1987a, b). The management of soft tissues is particularly thought to be responsible for affecting maxillary growth. The quantity and distribution of scar tissue after healing of the soft tissues seems to be the principal factor responsible for growth inhibition (Ross, 1987b).

To elucidate the effect of palatal surgery on maxillary growth, animal experiments have been carried out on different species. These studies sometimes show conflicting results. Sarmat (1958), for example, did not find facial growth inhibition after elevation and excision of a strip of palatal mucoperiosteum in Macaca rhesus monkeys. In a series of studies, Bardach and co-workers (1988, 1988) created unilateral soft and hard tissue defects in the lip, alveolus, and palate of rabbits and beagle dogs. Growth aberrations were found after surgical repair of the lip and palate, or after lip closure alone. Bardach (1990) concludes from his series of studies that lip closure results in an increase of lip pressure, which could be an important factor in craniofacial growth aberration. This is supported by Dixit et al. (1992) who, using the same experimental design, found that early repair of palatal defects in 8-week-old beagles, with oral mucosal or mucoperiosteal flaps, did not inhibit maxillary growth. These animals, however, were followed for only 12 weeks rather than to the end of the growth period, nor was the shedding of the teeth completed at the end of the study.

On the other hand, Herfert (1954, 1956, 1958) concluded from a split mouth study on five beagle dogs that the raising of palatal mucoperiosteum and the presence of denuded palatal bone after palatoplasty resulted in maxillary growth impairment. In a series of studies following Herfert's design, Kremenak et al. (1967, 1970) and Kremenak (1984) found that unilateral excision of a strip of mucoperiosteum adjacent to the posterior deciduous teeth resulted in inhibition of maxillary growth. The split mouth design has, however, several disadvantages.

In the studies of Wijdeveld et al. (1989, 1991), palatal surgery according to von Langenbeck, was simulated in beagle dogs of different ages in a nonbony cleft model. It resulted in a narrowing of the dental arch, which became apparent only after shedding of the teeth. This was in contrast with the arch development in control animals, where the posterior teeth tended to displace laterally during growth. Histologic evaluation showed that the composition of the scar tissue in the experimental groups remained different from the normal mucoperiosteum, irrespective of the age at which surgery was performed. The scar tissue covering the lateral wound areas...
adjacent to the posterior teeth lacked large blood vessels and elastic fibers and showed a mainly transversal orientation of collagenous fibers. The scar tissue was also attached to the underlying bone by Sharpey’s fibers. Furthermore, it was noticed that the mucoperiosteum was continuous with the periodontal ligament. These factors might result in a tensile force in a mediolateral direction on the teeth in dogs that were operated on. These authors suggested that prevention of scar tissue attachment to the underlying bone might lead to a more favorable dento-alveolar development.

Prevention of this attachment might be accomplished by separation of scar tissue and bone by implantation of membranes or by modifying the technique of palatal repair. Using the experimental model of Wijdeveld. In de Braeck (1992) attempted to prevent the development of Sharpey’s fibers with the use of biocompatible, biodegradable membranes. Premature degradation of the membranes influenced the results, but with improvement of those membranes the development of Sharpey’s fibers might be prevented. It remains to be elucidated whether or not growth inhibition could be prevented by membrane implantation.

Another way of preventing scar tissue attachment, namely, modifying the surgical technique, is the subject of this study. The aim was to compare the development of Sharpey’s fibers after two different techniques of palatal repair. Closure of a mucoperiosteal palatal cleft in beagle dogs was performed either by simulating the von Langenbeck technique or by using a modified partially split flap technique, by which denudation of palatal bone is avoided. The latter technique is a combination of a mucosal palatal flap technique as used by Perko (1974) and the von Langenbeck technique. The major palatine neurovascular bundle was displaced with the flap, in order to provide a good vascularization of the flap. This is in contrast with the mucosal palatal flap technique, in which the greater palatine artery remains in situ. For this reason, the risk of necrosis, which Perko (1974) considered one of the disadvantages of his technique, is decreased. The results of these techniques were evaluated histologically and compared with a control group.

METHODS

Animals

The experiments were performed on eight beagle dogs. In two dogs (age 6 months), palatal repair according to von Langenbeck was simulated as previously described by Wijdeveld et al. (1991). In four dogs (age 18 months), palatal surgery was performed using a partially split flap technique. Two young adult dogs served as controls. In all dogs the permanent dentition was completed.

Surgical Procedures

Prior to the surgical procedures, the animals were premedicated with 0.5 mL Thalamonal® (fentanyl, 0.05 mg/mL + droperidol 2.5 mg/mL; Janssen Pharmaceutica, Beerse, Belgium) and 0.5 mL Atropine (atropine sulphate 0.5 mg/mL). Subsequently, they were anesthetized with an intravenous injection of 30 mg/kg Narcovet® (sodium pentobarbital 60 mg/mL; Apharmo, Arnhem, The Netherlands). After intubation, anesthesia was maintained with Ethrane® (enflurane 15 mg/mL; Abbott, Amstelveen, The Netherlands).

The oral mucosa and the dentition were cleaned with chlorhexidine digluconate 1% in water. In addition approximately 6 mL Xylocaine® (lidocaine hydrochloride 0.4 mg/mL + adrenaline 0.0125 mg/mL; Astra Chemicals, Rijswijk, the Netherlands) was injected into the palatal mucoperiosteum to avoid excessive bleeding during surgery.

In all experimental animals, a soft tissue defect was created in the medial region of the palate by incising, elevating, and removing an elliptically-shaped mucoperiosteal flap. This flap extended from a line just behind the canines to the dorsal margin of the hard palate. The maximum width of the flap was one-third of the transverse distance between the fourth premolars.

In the dogs in which palatal repair according to von Langenbeck was simulated, relaxation incisions were made on both sides of the palate adjacent to the posterior teeth, and the remaining palatal mucoperiosteum was elevated from the underlying bone with a small raspatory. The major palatine neurovascular bundle was not damaged during the operation. The soft tissue defect was closed in the midline and sutured in one layer with 4-0 Vicryl, leaving two areas of denuded bone adjacent to the dentition (Fig. 1).

In the dogs in which the defect was closed using a partially split flap technique, the mucoperiosteum was elevated from the medial side of the bone to localize the greater palatine foramen and the major palatine neurovascular bundle in order to prevent its damage during surgery. On both sides of the palate adjacent to the posterior teeth, the mucoperiosteum was incised for half of its thickness. These incisions reached from canine to permanent second molar. Horizontal cleavage of the mucoperiosteum was then performed from the lateral side in the

![FIGURE 1 Schematic drawing of the simulated von Langenbeck technique resulting in denuded bony areas.](image-url)
medial direction, until the split area was at least half the size of the soft tissue defect in the midline. On the medial side of the split area, a vertical incision was made in the lower layer of the mucoperiosteum reaching to the bone. Subsequently, the mucoperiosteum was mobilized and replaced medially, and the soft tissue defect was closed and sutured in one layer with 4–0 Vicryl. The areas of the palate adjacent to the denudation thus remained covered with the bone-related layer of the mucoperiosteum (Fig. 2).

All experimental animals were medicated preoperatively with 1 mL of the antibiotic Alshipen® 15% (ampicillin anhydride 150 mg/mL; Mycofarm, de Bilt, The Netherlands) and maintenance doses of 1 mL Alshipen® LA (ampicillin anhydride 100 mg/mL; Mycofarm, de Bilt, The Netherlands) were given the 2nd and 4th day postoperatively. All animals received a normal diet after surgery.

Histologic Processing

For the histologic evaluation, all animals were killed 12 weeks after surgery. Prior to perfusion, the animals were brought under general anesthesia using 30 mg/kg Narcovet after which 0.5 mg/kg Thromboliqine® (heparin; Organon

FIGURE 2 Schematic drawing of the partially split flap technique. After surgery no denuded bone is present.

FIGURE 3A and B Border between the palatal bone and the mucoperiosteum in a control animal. The mucoperiosteum is connected to the bone by a few thin Sharpey’s fibers. B = bone, M = mucoperiosteum, SF = Sharpey’s fibers, BV = blood vessel. (a) Hematoxylin and eosin, ×320; (b) polarization microscopy of area 3a, ×320.
FIGURE 4A and B  Border between the palatal bone and the mucoperiosteum in an animal in which palatal repair according to von Langenbeck is simulated. The mucoperiosteum is attached to the bone by thick Sharpey's fibers. B = bone, SF = Sharpey’s fibers, S = scar tissue. (a) Hematoxylin and eosin, ×350; (b) polarization microscopy of area 4a, ×350.

Teknika, Boxtel, The Netherlands) was administered. After some minutes a lethal dose of Narcovet was injected intravenously. The thorax of the animal was opened and the vascular system was perfused with physiologic saline via the arch of the aorta, followed by 4% neutral formaldehyde as a fixative.

After perfusion, the maxillae were dissected and immersed in 4% neutral formaldehyde for another 2 weeks. Then, they were sawed into five smaller blocks. Two blocks contained the left and right second premolars and the adjacent lateral palatal areas. Two other blocks contained the third premolars including the palatal areas. The last block contained the midpalatal area between the fourth premolars and the first molars. The blocks were decalcified in 20% formic acid and 5% sodium citrate, dehydrated and embedded in Paraplast® (Monoject Scientific, Athy, Ireland). Serial frontal sections of 7 μm were prepared. For general tissue survey, sections were stained with hematoxylin and eosin. Five sections from each block with a spacing of 175 μm were stained according to Goldner’s modification of Masson’s staining for collagenous fibers (Burck, 1973), Herovici’s (Herovici, 1963) polychrome staining for the study of young (Type III) and old (Type I) collagen (Levame and Meyer, 1987), and the Weigert-Van Gieson (Lillie, 1965) staining to study elastic fibers. All histologic observations were carried out by two independent observers. A 4-point semi-quantitative scale (none, few, moderate, many) was used to categorize the number of Sharpey’s fibers.

RESULTS

In the control animals, the palatal bone was covered with stratified squamous epithelium which was parakeratotic. Many villi protruded into the underlying connective tissue. Just underneath the epithelium, the fibrous connective tissue, containing many coarse collagen Type I fibers, formed a three-dimensional network. In the deeper layers of the connective tissue, sagittally-oriented collagenous fibers became more predominant, and elastic fibers were randomly distributed. Here an expansion tissue was present with sagittally-oriented large blood vessels along the whole width of the palate. Between those blood vessels, the major palatine artery and branches of the palatine nerve were found at the lateral aspect close to the bone. The periosteal part of the mucoperiosteum consisted of a thin
layer with some resting osteoblasts. Only a few thin collagen Type I fibers connected the fibrous layer of the mucoperiosteum to the palatal bone (Fig. 3). The palatal bone was of the lamellar type, no deposition was found. The palatal mucoperiosteum was continuous with the periodontal ligament; cervical periodontal fibers were fanning out into the gingiva and the deeper layers of the palatal connective tissue. In the more apical region of the socket these fibers connected the tooth to the alveolar bone. Neither deposition nor resorption of alveolar bone was found.

In the animals, in which palatal repair according to von Langenbeck was simulated, scar tissue was found in the lateral operation areas and in the medial suturing area. In these areas, the epithelium seemed to be somewhat thinner and lacked villi, the connective tissue showed thinner, probably Type III, collagenous fibers and the absence of elastic fibers was evident. The structure of the mucoperiosteum and the palatal bone in other areas was similar to the control animals. The system of blood vessels and the major palatine arteries and nerves were displaced medially with the displaced mucoperiosteum. A very thin osteogenic layer covered the palatal bone in most places. It consisted of a few inactive osteoblasts and some thin collagenous fibers inserted into the palatal bone.

The mucoperiosteum and the bone in the scar tissue areas, however, were different. In those areas many thick collagen Type I fibers penetrated into the palatal bone as Sharpey’s fibers, thus creating an attachment of the scar tissue to the bone (Fig. 4). The periodontal ligament showed an attachment between the teeth and the gingiva, the mucoperiosteal connective tissue, and the alveolar bone.

In the dogs in which a partially split flap technique was used, the superficial mucoperiosteal layer in the lateral operation areas consisted of scar tissue. The deeper palatal layer appeared to be normal. In the medial incision area, scar tissue was also found. The epithelium in the scar tissue areas seemed to be somewhat thinner and it lacked villi. A layer of scar tissue was also found between the deeper and the displaced superficial layers of the mucoperiosteum. The demarcation of the scar tissue was, however, rather vague (Figs. 5 and 6). The structure of the mucoperiosteum in other areas was similar to the control animals (Fig. 7). The system of large blood vessels and the major palatine arteries and nerves were displaced medially, however, large blood vessels were still present in the deeper layer in the lateral operation areas. The palatal bone was of the lamellar type without resting lines. A very thin osteogenic layer covered the palatal bone in most places. It consisted of a few inactive osteoblasts and the fibrous layer was connected to the palatal bone by few thin collagen Type I fibers. The periodontal ligament of the teeth adjacent to the lateral operation areas did not differ from the other groups.

**Discussion**

Healing of palatal mucoperiosteum after two different techniques of palatal surgery was studied histologically and compared with controls. The presence of a bony palatal cleft was
The situation is comparable to the control animal if bone is unconsolidated. (a) Hematoxylin and eosin, x 320, (b) polarized microscopy, of the femoral neck region. A question mark and a dotted line indicate the zone of unconsolidated bone, adjacent to the cortical bone. The histological findings are consistent with the findings in the intact femur. (c) Polarized microscopy, of the femoral head region. The bone is unconsolidated, and the bone is not resorbed. The histological findings are consistent with the findings in the intact femur. (d) Polarized microscopy, of the femoral head region. The bone is unconsolidated, and the bone is not resorbed. The histological findings are consistent with the findings in the intact femur.

The situation is comparable to the control animal if bone is unconsolidated. (a) Hematoxylin and eosin, x 320, (b) polarized microscopy, of the femoral neck region. A question mark and a dotted line indicate the zone of unconsolidated bone, adjacent to the cortical bone. The histological findings are consistent with the findings in the intact femur. (c) Polarized microscopy, of the femoral head region. The bone is unconsolidated, and the bone is not resorbed. The histological findings are consistent with the findings in the intact femur. (d) Polarized microscopy, of the femoral head region. The bone is unconsolidated, and the bone is not resorbed. The histological findings are consistent with the findings in the intact femur.
from the periosteum, allowing the greater palatine artery to remain in situ. This means that the risk of necrosis, which Perko (1974) considered one of the disadvantages of the palatal mucosal flap technique, is reduced with the partially flap technique.

The present results indicate an absence of Sharpey’s fibers after palatal repair using a partially split flap technique in contrast to palatal repair simulated according to von Langenbeck. Furthermore, the palatal bone, after palatal repair using a partially split flap technique, is completely normal. Whether this will have a beneficial effect on dento-alveolar development remains to be investigated. As the present study shows prevention of the development of Sharpey’s fibers is possible, a longitudinal study on dento-alveolar development in beagle dogs operated at the age of 12 weeks, is currently in progress.

REFERENCES


Commentary

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Generally speaking, I do not have much to say regarding this comparison of corrective surgical techniques. The use of fentanyl as a premedicant, and pentobarbital for general anesthesia is satisfactory, although better methods are available. Analgesia with pentobarbital is not good, but this is compensated for by the powerful analgesic effect of the fentanyl.

This brings us to the next point which concerns postoperative analgesia. This type of surgery involves a considerable amount of pain and discomfort postoperatively, and yet no mention is made of an analgesic regime. I would regard this as a serious omission because it casts doubt on the postoperative care (which is not mentioned).

The use of lidocaine to control excessive bleeding is not a technique with which I am familiar. We use it as a local anesthetic and, at this concentration, for perfusion purposes. The adrenaline component of the mixture will cause vasoconstriction, which is there to delay the systemic absorption of the local anesthetic. This will probably help to control bleeding.

Postoperative care should be described, including postoperative analgesia. I question the use of a local anesthetic for hemostasis. This should be explained more precisely.

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PLEASE NOTE: As a follow-up to the above commentary, the authors selected to add two paragraphs to the original text, as is shown in italics. They also responded with the following comments:

Author Response

Thank you for sending the comments of Dr. D.H. Neil on our manuscript: “Wound Healing in Beagle Dogs after Palatal Repair without Denudation of Bone.”

The point raised by Dr. Neil, that after this type of surgery, the animals will suffer considerably from pain and discomfort, is not in agreement with our experience. It surprised us, and the veterinarian of our animal laboratory, how fast the animals recovered. A few hours after surgery, they ate and drank normally, they were attentive and came inquisitively forward, wagging their tails if someone approached their cages. So, we decided that postoperative analgesia was not indicated. Dr. Neil is right in his description of the working mechanism of local anesthetics. In oral surgery, especially in cleft palate repair, local anesthetics containing adrenalin are routinely used to avoid bleeding during surgery, just because of the vasoconstriction provoked by the adrenalin component of it. In palatal surgery in dogs, the use of vasoconstrictors is essential due to the very rich vascularization of the palate, and it helps to control bleeding during surgery.

Yours sincerely,

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