Facial Growth in the Rabbit After Autologous Grafting in Unilateral Clefts

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In each of 39 rabbits, a left-sided cleft of the lip and alveolar part of the maxilla were made by removal of the premaxillomaxillary suture. In 5 animals, the cleft was left unrepaired. In 12 animals, an autologous graft from the mandibular symphysis was introduced, and in 12 animals autologous rib-growth cartilage was used for grafting. Lateral photographs of adult skulls in a standardized position were made, and a computerized craniometric analysis of the position of 13 anatomic landmarks in an orthogonal coordinate system was performed. The data from the grafted skulls (12) were compared with those from the control series (20) and subjected to a multivariate analysis, according to Hotelling.

Comparison of the grafted adult skulls with 20 unoperated controls and the animals with unrepaired artificial clefts revealed that introduction of rib-growth cartilage leads to an improvement with respect to the growth of the facial skeleton. The initial orientation of the graft in the cleft appeared to be important for its integration in the jaw.

KEY WORDS: cleft repair, facial growth, growth cartilage, graft, rabbit.

In children with unilateral cleft lip and palate, the lip is repaired early in postnatal life, at ages varying between 6 weeks and 3 months. As to the alveolar clefts in the maxilla, there is no agreement on the best therapeutic approach: a few authors (e.g., Enany, 1981; Rosenstein et al, 1982) recommend primary osteoplasty, but the majority prefer secondary bone grafting (Boyne and Sands 1972; Rune et al, 1980; Ahlholm et al, 1981; El Deeb et al, 1982) or secondary periosteoplasty (Hellquist et al, 1983). The effects of surgical repair and bone grafts on midfacial growth are contradictory. Most results point to growth retardation of the repaired side (Rehrmann et al, 1970; Friede and Johanson, 1972), but some authors (Robertson and Fish 1972; Nylen et al, 1974) do not report growth retardation after implanting bone grafts.

An explanation for disappointing results of early bone grafting might be that this tissue has no capacity to grow after implantation. Facial growth is continuing, and perhaps the graft influences growth in the region of the cleft through either inhibition or stimulation. Kluzak (1972) introduced transplantation of rib-growth cartilage. He demonstrated that a rib graft taken from the fifth or sixth osteocartilaginous junction increased in size when placed in position either with or without a functional load. He mentioned the application of this method in children with clefts. Considering clinical application, care should be taken so that interference with normal function is as small as possible. It is questionable whether the donor site in Kluzak’s approach is the best possible choice.

The present study also investigates the effect of introduction of autologous tissue. However, instead of a bone graft, tissue with an inherent capacity to grow was implanted into an artificial
cleft. For donor sites we used part of the mandibular symphysis as well as the ventral extremity of the floating ribs. The rabbit was used as experimental model.

MATERIALS AND METHODS

Fifty-nine New Zealand white rabbits were divided into the following groups:

Group I: Twenty animals, unoperated controls (Fig. 1A).

Group II: Five animals with a surgically made left-sided cleft of lip and alveolar part of the maxilla; the lip was closed, the cleft in the jaw remained unrepaired (Fig. 1B).

Group III: Twelve animals with a surgically made left-sided cleft of lip and alveolar part of the maxilla; the lip was closed, and an autologous graft from the mandibular symphysis was introduced into the defect of the jaw.

Group IV: Twelve animals with a surgically made left-sided cleft of lip and alveolar part of the maxilla; the lip was closed and the defect in the jaw was filled with autologous rib-growth cartilage that included the osteocartilaginous junction, the periosteum, and the perichondrium. The graft was taken from the left floating ribs.

After the results in Group IV were studied, two small groups were added:

Group V: Five animals in which the rib transplant was placed parallel to the direction of the cleft (Fig. 1C).

Group VI: Five animals in which the rib transplant was placed perpendicular to the direction of the cleft, the cartilaginous part pointing forward. To fill the cleft satisfactorily, the graft had to be split longitudinally (Fig. 1D).

Creation of the cleft and grafting were performed in one operation. All animals were operated on at the age of 4 weeks, hence shortly after weaning, under appropriate anesthesia, using 0.6 ml/kg acepromazine as premedication and 0.2 ml/kg pentobarbital sodium as anesthetic. Moreover, xylocaine with epinephrine was applied as a local anesthetic in the region of the upper lip and jaw.

The cleft in the alveolar process was made by removal of the vertical part of the premaxillomaxillary suture, using a dental bur; careful attention was paid to leaving the facial nerves and the nasal mucosa undamaged. In all cases, the soft tissues and the lip were closed by suturing the layers according to the principle of Veau (1931).

The animals were allowed to grow to maturity. At the age of 24 weeks, they were sacrificed, body weight was registered, and the skulls were prepared. They were described macroscopically, and a computerized cranio-metric analysis was performed. For this purpose, lateral photographs were taken of the skulls in a standardized position. On the prints,
13 anatomic landmarks were identified. The coordinates of these points were recorded automatically in an orthogonal coordinate system. Correction for differences in size of the skulls was performed by expressing the coordinates as a percentage of the distance between the caudal point of the synchondrosis sphenooroccipitalis and the cranial point of the sutura lambdoidea (lambda). Verwoerd-Verhoef (1974) and Urbanus (1974) demonstrated that this distance was not changed by the experiment and, therefore, could be accepted as reference.

The data obtained in the experimental series were compared with those of control series and tested for statistical significance by applying the Hotelling multivariate analysis. The results are presented in a series of illustrations that show the degree of growth retardation of the affected side of the skulls.

RESULTS

Surgical Procedures

Altogether, 55 rabbits were operated. Sixteen animals died of infections that were unrelated to the experiments. These deaths were about equally distributed throughout the groups and are not included in the 59 animals mentioned in the Materials and Methods section.

The body weights of the operated and the control animals did not differ significantly at the age of 24 weeks, indicating that there was no irreversible retardation of general growth because of the operation. In all animals, the continuity of the bone of the facial skeleton was restored, remnants of the cleft being visible only in cases belonging to Group II, in which no graft was introduced. As to the donor sites, complete healing occurred in all cases. In one case, a local infection, which was treated with antibiotics, developed.

Facial Growth in Animals with Unrepaired Cleft

Macroscopic study of the skulls of animals with unrepaired clefts revealed a distinct asymmetry of the face, caused by deviation and rotation of the premaxillary bone and deviation of the nasal bone. Remnants of the cleft are visible in three out of five animals. The molar complex is also asymmetrical, as can be seen in the diagram based on the average values of the craniometric measurements (Fig. 2).

Facial Growth after Grafting of Symphysial Cartilage

Macroscopic study of the skulls after grafting symphysial cartilage revealed in all cases deviation of the nasal bone to the left side, and in eight of the 12 animals, a clearly visible asymmetry of the molar complex. In Figure 3, the diagram is presented. There was definite growth retardation of the operated left side of the facial skeleton, resulting in asymmetry of the snout. The differences between the values of the coordinates of the anatomic landmarks, especially those that reflect the position of the nasal part and the molar complex in this group and in the controls, were statistically significant ($p < 0.01$).

In all cases, a rough, bony surface at the site of the healed cleft was observed. The mandibles did not show any deviant growth, neither was the eruption of the lower incisors disturbed. The results of this approach are not improved over those in Group II (see Fig. 2), in which no graft was introduced into the cleft.

Facial Growth after Grafting of Rib-Growth Cartilage

Macroscopic observation suggested that after grafting rib-growth cartilage, the growth results of this method were better than in the series with mandibular grafting, particularly with respect to the position of the molar complex. In agreement with this impression, we found that there are no statistically significant deviations from the control values of the measurements. Figure 4 shows that the values for the experimental animals are nearly the same as for the controls.

As to the graft, it was surprising that in eight of the 12 cases a rib fragment could be recognized. The graft had increased in size, and ossification had also occurred. In some skulls, growth of the graft had proceeded in an unintended direction, resulting in peculiar exostoses on the outside of the maxilla (Fig. 5).

Based on the impression that the results might be influenced by the direction in which the graft had been introduced, an additional experiment was carried out. In five animals (Group V), the graft was placed parallel to the direction of the cleft with the cartilaginous part pointing upward to the nasal bone, whereas in another five rabbits the graft was placed perpendicular to the direction of the cleft, the cartilaginous part pointing forward, in the direction of growth of the facial skeleton. The results are shown in Figures 6 and 7. The diagrams indicate that the asymmetry in the animals of Group V is less than in those of Group VI, but the difference in asymmetry is not statistically significant.

Macroscopic examination revealed that a
grown and ossified rib fragment could be identified in four of five skulls of Group V. In all cases, one or more exostotic outgrowths were found. No remnants of the graft could be observed in Group VI.

**DISCUSSION**

**Animal Model**

In 1974, the rabbit was introduced in the study of growth in cleft palate by Verwoerd-Verhoef. As to the clefts, the situation is not similar to the congenital clefts in children. In rabbits, a cleft can be made only after weaning, i.e., at the age of 4 weeks. It is not possible to create a cleft as a birth defect. Verwoerd-Verhoef concluded that the growth abnormalities, which occurred in this animal after experimentally creating clefts, were similar to those described in skulls of untreated patients (van Limborgh 1964). Urbanus (1974) used this model to compare the growth results of three surgical methods and concluded that the method according to Veau (1931), i.e., suturing the soft tissues without bone transplantation, gave the most satisfying growth, but that growth of the operated side was retarded, leading to asymmetry of the face. The same animal model was used by Bardach and his co-workers (Bardach and Eisbach 1977; Eisbach et al, 1978; Bardach et al, 1979a; Bardach et al, 1979b; Bardach et al, 1980). Recently, there has been some criticism of the use of rabbits for this purpose, because of the different patterns of growth and mastication of this species (Sarnat 1982). Bardach et al (1982) and Bardach and Mooney (1984) also studied beagles, in which cranio-

**FIGURE 2** Averaged, normalized position of the anatomic landmarks on the left side of the skull. Dotted line, Group I (controls); solid line, Group II (operated; only lip closed). 1, synchondrosis spheno-occipitalis; 2, lambda; 3, bregma; 4, nasion; 5, spina nasalis anterior; 6, dorsal rim of the alveolus of the dorsal incisivus; 7, ventrocranial point of the alveolar part of the first premolar; 8, ventrocaudal point of the first premolar; 9, dorsocaudal point of the second molar; 10, dorsocaudal point of the alveolar part of the second molar; 11, spina masseterica; 12, dorsal termination of the sutura temporozygomatica; 13, foramen opticum.
facial growth is similar to growth in primates. However, they found the same retardation of facial growth following reconstructive surgery as they observed in rabbits.

Although we certainly agree in general with the objections against use of lagomorphs as an experimental model for the study of abnormalities in man, we feel that the previous results obtained in rabbits give sufficient support to continuation of this approach. We made the alveolar cleft by removing the premaxillomaxillary suture, according to the method developed by Verwoerd-Verhoef (1974) when the animals were 4 weeks old. Bardach et al (1980) did not perform experiments before the animals reached the age of 7 weeks; also, they created the cleft between the incisor and the premaxillomaxillary suture. In spite of these differences in experimental approach, their data demonstrated growth abnormalities similar to those we found. Their results as well as ours strongly suggest that it is the combination of disruption of the continuity of the skeleton and repair in the fast-growing facial skull that is responsible for the growth retardation.

Grafting

In their treatment of clefts in patients, most authors transplant spongy bone from the iliac crest. This procedure appears to be successful in combination with subsequent orthodontic treatment (Bergland et al, 1986). A problem with this approach is that the graft neither grows itself nor stimulates the growth of the surrounding bone; so this method cannot be applied be-
before facial growth is more or less completed. In the clinical setting, this means that one has to wait until the child has reached the age of 7 years.

In our study, we explored the possibility of early grafting, using a graft with an inherent capacity to grow. The mandibular symphysis was chosen because of the common origin of this bone and the maxilla from the first branchial arch. It was presumed that this common origin might facilitate the acceptance and subsequent development of the graft (Bosker and van Dijk, 1980). Removal of a part of the mandibular symphysis was possible without interfering with normal function and without impeding the eruption of the lower incisors. At the age of 4 weeks, however, the animal’s symphysis appeared to contain only a small amount of cartilage, the ossification being already in an advanced stage (Hirschfeld et al, 1977). Besides, data in the literature do not point to the human mandibular symphysis as an important growth center. These factors are perhaps responsible for the unsatisfying growth results.

Kluzak (1972) reported that a rib graft grows independently of the acceptor site. We also found that the rib graft will grow and develop according to its own pattern, but only if it is placed parallel to the cleft.

Comparing the diagrams, it is obvious that the best results were obtained following implantation of rib tissue. However, the preservation...
of the characteristics of a rib in this series gave rise to other problems, such as exostoses. No exostoses were seen if the rib graft was introduced perpendicular to the cleft. In the adult skulls of this group we could find no remnants of the rib, suggesting that the rib tissue was integrated in the jaw. An explanation for these findings might be that in this approach at least two parts of a graft had to be introduced in order to fill the cleft, and therefore the graft had to be split. Damage to the periosteum and perichondrium must have occurred. Zins et al (1984) reported that preservation of the periosteum is required to prevent disintegration of the graft. Therefore, it seems likely that the partial absence of periosteum and perichondrium in case of the split graft has facilitated resorption.

FIGURE 5 Lateral view of the facial skull of rabbit belonging to Group IV. Rib fragment (*) and exostoses (**) are shown.

FIGURE 6 Averaged, normalized position of the anatomic landmarks on the left side of the skull. Dotted line, Group I (controls); solid line, Group V (operated, autologous graft from rib-growth cartilage, orientated in the direction of the cleft). For explanation of the landmarks, see Figure 2.
In summary, we conclude from our study that early grafting of rib-growth cartilage presents possibilities to improve the growth of the face, but more research is needed before the results of laboratory experiments can be used for therapeutic goals in children with clefts.

REFERENCES


