CARTILAGE REGENERATION IN THE SEPTAL AREA AFTER SUBMUCOUS CARTILAGINOUS RESECTION IN THE YOUNG RABBITS

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SUMMARY : The present work was carried out to study the morphological alterations in the fibrous layer of the perichondrium remaining "in situ", after subperichondrial resection of the septal cartilage, depending on the time of the cartilaginous repair. In these conditions, after granulation tissue formation, both neochondrogenesis and connective tissue differentiation were observed. Neochondrogenesis was found in defect areas next to the resection borders, and connective tissue formation in those further away. As a conclusion, we were able to show that a nearly complete regeneration of cartilage occurs from the cut ends of septal cartilage (complete resection of quadrilateral cartilage), and this is associated also with significant areas of cartilage regeneration from the perichondrium or surrounding connective tissue.

***Key Words :** Neochondrogenesis, Perichondrium, Rabbit.*

INTRODUCTION

Submucous resection is a satisfactory operation when performed by the appropriate techniques (10). Traditionally it is believed that after submucous cartilaginous regeneration is produced (5). In these conditions, angulations or deformities of the affected septal space have been found, at the expense of neoformed chondroid tissue (4). Recently, partial cartilaginous regeneration of resected septal cartilage has also been shown through experimental procedures (1, 4, 5, 10, 11, 13, 14). But, except Lopez-Aguado et al (5), nobody used the electron microscopic techniques in their studies. In addition, these authors were not able to protect the inner layer of perichondrium during the cartilagenous resection.

The purpose of the present study was to investigate the events during repair period after submucous cartilage resestion in rabbits at light and electron microscopy levels.

MATERIALS AND METHODS

This study has been carried out between 1993 and 1994 in GATA Research Center Microsurgery Laboratory. Twelve 12-to 18-week-old male and female New zeland white rabbits (3-3.5 kg, weight) were used. The animal received a general anesthesia with ketamine in a dose of 100 mg/kg supplemented with chlorpromazine in a dose of 5 mg/kg body weight, both administered i.m. A midline incision was made down the dorsum of the snout; subcutaneous haemostasis was established with electrocoagulation. The operation was continued under a surgical microscope. Using a 2 mm. fissure burr, one mm of nasal bone was removed from each side of the internasal suture, extending from the caudal end of the cartilaginous septum, up to the frontona-

sal suture level. Once the inner subperichondrial plane was reached on each side of the septal cartilage, an almost complete resection of the quadrilateral cartilage was carried out. Great care was taken to avoid involving the bone components of the septum. In all the cases, the removed cartilage was obtained for histological studies. After checking for complete haemostasis, the incision was closed with 4/0 silk suture. The animals were given 200 000 IU benzyl-penicillin and 250 mg dihydrostreptomycin for 3 days postoperatively. The rabbits were sacrificed under general anaesthesia at days 5, 10, 20 and 55 postsurgery, and the experimental area, including the remaining cartilage, was removed for light and electron microscopy (5).

Tissues for light microscopy were fixed in a 10 % formalin solution. Paraffin sections were prepared and stained with hematoxylene-eosin. Specimens for electron microscopy were fixed in 2.5% gluteraldehyde and were postfixed in 1 % osmium tetroxide in phosphate buffer. Following dehydration tissues were embedded in Araldite CY 212. Semi-thin and thin sections were prepared from the blocks on a LKB Nova Ultramicrotome. Semi-thin sections were then stained with 1 % toluidine blue. Paraffin and semi-thin sections were examined and color pictures were taken at Olympus BHS/BHT light microscope. Thin sections were stained with aqueous uranyl acetate and Reynold's solution and examined and photographed with Zeiss EM 9S2 electron microscope.

RESULTS

On the 5th postoperative day, an inflammatory infiltrate of polymorphonuclear leukocytes and macrophages appeared in the resected area. On the tenth day, the tissue defect was filled with granulation tissue. Different structural zones were observed in the following days.

Fifty-five days after the septal cartilage excision, in the areas next to the resection borders, new cartilage appeared, while in those further from these borders fibrous tissue developed (Fig 1, 2A, B, 3A, B). The reaction of the cartilage resection includes proliferation and production of a large quantity of fibroblasts migrating into the exudate-filled space and invading the cartilage through the crevices. The mitotic activity of the viable cartilage and the migration and proliferation of fibroblasts exerting from the surrounding, thickened and unravelled perichondrium, underline the reactive changes in these tissues (Fig 1).

The morphology of newly formed and original septal cartilage remains quite different. The new cartilage consists of smaller chondrocytes with less intercellular substance and without any obvious orientation. Frequently blood vessels can be observed to run through the new cartilage. The original septal cartilage is avascular and characterized by larger cells with a greater intercellular distance and an orientation in rows. The demarcation between the new cartilage and original septal cartilage as a rule is sharp (Fig 1).

Fig - 1 : Fifty - five days after partial resection of septal cartilage (S). Clusters of viable and very active cells (C). Fibroblasts migrate into the crevices to remove necrotic material (asterisks). E : Exudate, b : Bone, Ep : Epithelium, P : Perichondrium, arrowhead : Vessels (Hematoxylene - Eosin, X48).

The transition zone between both types of newly-formed tissues showed intermediate characteristics of cartilage and fibrous connective tissue (Fig 3A, B).

Ultrastructurally, the cells of the new cartilage next to the resection borders presented some characteristics that were large lipid droplets, short superficial projections and intracytoplasmic filaments (Fig 4, 5).

DISCUSSION

Nasal septal surgery in children remains a controversial subject because of its possible detrimental effect on the subsequent growth of the mid-facial skeleton and also because of the associated increase of nasal saddling. Following Hayton's (3) careful study of 31 children who had undergone Killian's

Fig - 2A : Newly-formed cartilage tissue (N) in septal defect border.

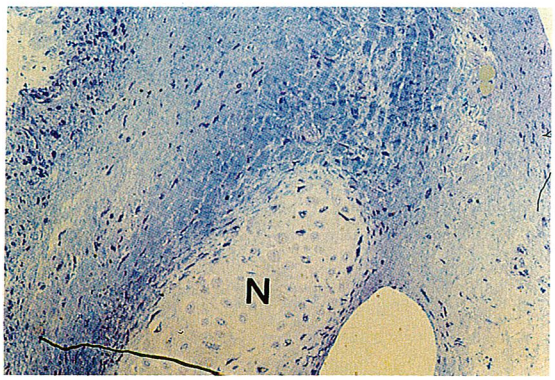

Fig - 3A : New formation of cartilage (N) 55 days after partial resection of cartilaginous septum is observed in semi-thin section (Toluidine blue, X240).

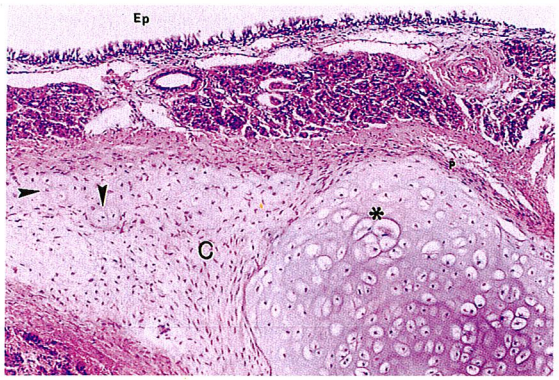

Fig - 2B : In higher magnification, the ingrowing cells from the perichondrium (P) differentiate into chondroblasts (C). Chondrocytes in lacunae (asterisk). The viable cells from islands of cartilage (arrowheads). S : Septal cartilage, Ep : Epithelium, E : Exudate (Hematoxylene-Eosin, A:X48, B:X120).

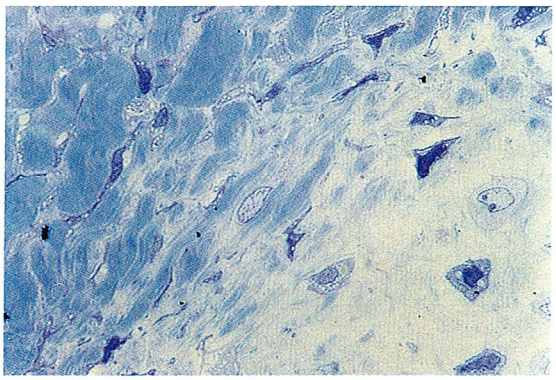

Fig - 3B : In higher magnification, transition zone between both newly formed tissues in septal defect : cartilage and fibrous tissue. Cells with intermediate characteristics of cartilage and fibrous connective tissue are observed (Toluidine blue, X1200).

submocous resection, a third of whom developed nasal saddling and broadening, a more conservative approach was generally adopted, delaying surgery till the age of 16 years.

Encouraged by clinical and experimental studies, others have challenged this concept. Fuchs (2) demonstrated that some cartilagenous regeneration and limited osteogenesis occured in young rabbits following septal surgery. Bernstein (1), in his experimental study, found that submucous resection in young pups was followed by normal facial development and stressed the critical importance of preserving septal mucoperichondrium. More recent experiments, however, have shown that, even with preservation of mucoperichondrium (4, 7, 9), resections of cartilage have caused retardation of nasal growth.

Jeffries and Evans (4) demonstrated that on the 21st post-operative day, coronal sections showed that already there was a downgrowth of new cartilage from the cut surface of the dorsal supporting cartilage. They suggested that cartilaginous regeneration occurs from both the cut ends of the septum and from the mucoperichondrium, and that this is asso-

Fig - 4 : Transmission electron micrograph of transition zone. Neochondrocyte (arrow) and chondrocytes (arrowheads) are observed (Uranyl acetate and lead citrate, X6000).

Fig - 5 : Ultrastructural characteristics of a neochondrocyte (N) with a large lipid droplet (thick arrow), short superficial projections (arrowheads) and intracytoplasmic filaments (thin arrow), (Uranyl acetate and lead citrate, X15000).

ciated also with significant areas of cartilage regeneration from the perichondrium.

The behaviour of injured septal cartilage and the reaction of traumatized perichondrium in young growing rabbits were described earlier (12, 13, 15). The observations showed that the perichondrium has a great capacity to heal without scarring and can easily produce new cartilage (15). Former experiments in which a submucous resection of the middle third of the septal cartilage was immediately followed by reimplantation of the cartilaginous strip in the orginal surgery, deviations occur which will increase during further development (6, 15).

We studied the morphological alterations in the fibrous layer of the perichondrium remaining "in situ", after subperichondrial resection of the septal cartilage, depending on the time of the cartilaginous repair. In our attempts to dissect the septal cartilage, it has been possible to achieve a seperation of the cartilage from the innermost layer of the perichondrium. But, in a similar study Lopez-Aguado et al (5) were not able to separate the cartilage from the inner layer of perichondrium (also termed basal or chondrogenic).

In all cases of this experiment varying amounts of neocartilage were formed. The neocartilage may thus originate from : (a) surrounding connective tissue; (b) cells located in the inner or outer layers of the perichondrium and (c) migrating cells of the basal layer in the perichondrium of the remaining cartilage.

The regenerated cartilage remains morphologically different from the original septal cartilage. Even in the adult animals the regenerated cartilage consists of relatively small chondrocytes without any columnar arrangement (13). A similar type of new cartilage has been described by Pirsig (8) in the septa of children who had a nasal trauma or surgery some years before. He considered this to be young cartilage, not fully differentiated.

The presence of transitional zones between cartilage and fibrous connective tissue with cells showing an intermediate phenotype between chondrocyte and fibroblasts, has also been described in a previous study (5). These transitional cells provide greater evidence that new chondrocyte and fibroblasts growing after septal cartilage removal originate largely from a common precursor cell either in the fibrous perichondrial layer or in the connective tissue surrounding it (5).

Neochondrogenesis in the resection borders may be produced by biochemical stimulation governed by the remaining cartilage or its perichondrium. In addition, mechanical forces were reported to contribute to the cartilaginous differentiation (5). In that case the septal space lacking its cartilaginous support, suffers from constant movement during normal respiration. In a study, the mechanical action was reported to favour the processes of cartilage neoformation on the resection borders and of fibrous tissue differentiation in the defect central areas (5).

As a conclusion, we were able to show that a nearly complete regeneration of cartilage occurs from the cut ends of septal cartilage (complete resection of quadrilateral cartilage), and this is associated also with significant areas of cartilage regeneration from the perichondrium or surrounding connective tissue. All these research have shown that it is very important to protect the perichondrium in septoplasty and especially submucosal resection.

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