CELAPURE

EFFECTS OF HUMAN AMNIOTIC FLUID ON COSTAL CARTILAGE REGENERATION (AN EXPERIMENTAL STUDY)
Effects of Human Amniotic Fluid on Costal Cartilage Regeneration (an Experimental Study)

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Abstract

Objective: After surgical correction of thoracic wall deformities, promoting neochondrogenesis in the perichondrial bed is very important for obtaining a flexible chest wall. In this experimental study, we aimed to investigate the effects of human amniotic fluid on cartilage regeneration in the costal perichondrial bed in a rabbit model.

Methods: Fifty-four adult New Zealand rabbits were divided into three groups, with 18 rabbits in each group. The third and fifth costal cartilages were excised totally on the right side and partially excised on the left side in all groups. Group 1 served as controls. All rabbits in group 1 underwent closure of the perichondrium of the third costal cartilage and closure of the perichondrium of the fifth costal cartilage with reimplantation of reshaped cartilages. Rabbits in group 2 underwent closure of the perichondrium of the third and fifth costal cartilages after the administration of human amniotic fluid into the perichondrial bed. Group 3 rabbits received both human amniotic fluid and underwent cartilage reimplantation. The third and fifth costal perichondriums in group 3 rabbits were closed after the administration of human amniotic fluid and the reimplantation of reshaped cartilages. Rabbits were sacrificed at two, eight and 12 weeks after operation.

Results: Numerical scores for the right perichondrial bed were significantly higher for group 2 compared to group 1 (p < 0.05). But the difference was not significant for the left perichondrial bed (p > 0.05). The diameter of chondrogenesis also did not differ significantly between left and right perichondrial bed for all groups.

Conclusion: Our study shows that administration of human amniotic fluid into the perichondrial bed increases chondrogenesis in adult rabbits, an important finding which may contribute to improving chest wall flexibility after the surgical correction of pectus excavatum.

Introduction

Pectus excavatum (PE) is a congenital deformity of the chest wall with an incidence of approximately 1 per 300–400 live births [1]. The exact causes of PE are unknown but investigators have hypothesized that the deformity results from an unbalanced overgrowth in the costochondral regions. Correction of pectus excavatum deformities is done surgically. Surgical repair of PE is commonly based on extensive modifications of a procedure originally described by Brown [2], for example, the Ravitch procedure [3], the minimally open technique described as Erlangen procedure [4,5], or the minimally invasive method without costal cartilage resection as described by Nuss [6]. Some researchers have suggested that correction of PE should be considered a cosmetic procedure [7–9]. However, the findings of two recent meta-analyses, which examined the impact of surgical repair on cardiovascular function and pulmonary function in PE patients [10,11], were at variance with assertions that surgical repair is primarily cosmetic and results in only minimal physiological improvement.

The optimal age for the repair of PE is still controversially discussed in the literature. Pectus deformities seem to become more pronounced during puberty. Consequently, there may be a recurrence of PE if correction is performed before adolescence [12]. Currently, it is considered advisable to postpone PE repair until after puberty. In vitro studies have shown that hyaluronic acid (HA) and glycosaminoglycan have a regulatory effect on chondrocyte activity by increasing proteoglycan synthesis and secretion [13–15]. Human
amniotic fluid (HAF) contains high concentrations of high molecular weight HA and high concentrations of HA-stimulating activator (HASA) [16,17]. There can be no doubt that cartilage regeneration plays a fundamental role in maintaining chest wall flexibility after PE repair. However, cartilage regeneration in the perichondrium after the resection of deformed cartilages is not well-documented, and there is no data in the literature on how to improve cartilage regeneration. In this experimental study we investigated the effects of human amniotic fluid on cartilage regeneration in the costal perichondrial bed in a rabbit model.

Materials and Methods

Fifty-four New Zealand adult rabbits were used in this study. All procedures were performed in the Experimental Animal Breeding and Research Center of the Military Medical Academy of Gulehane. This experimental study was approved by the institutional ethics committee.

Experimental groups

Fifty-four adult New Zealand rabbits were divided into three groups, with each group consisting of 18 rabbits. Each of these groups was divided into three further groups, with six rabbits per sub-group. The third and fifth costal cartilages were excised totally on the right side and partially on the left side in all groups. Each group was evaluated at two, eight and 12 weeks after the operation. Group 1 was the control group. In group 1 rabbits, the third costal perichondriums were closed and the fifth costal perichondriums were closed after the reimplantation of reshaped cartilages. All rabbits in group 2 received human amniotic fluid (HAF) into the perichondrial bed with no cartilage reimplantation. The perichondriums of the third and fifth costal cartilages in group 2 rabbits were closed after the administration of 0.1 ml HAF into the perichondrial bed. Rabbits in group 3 received HAF and underwent cartilage reimplantation. The third and fifth perichondriums were closed after the administration of HAF and the reimplantation of reshaped cartilages. Costochondral junctions were preserved in the all partial and all total resections of cartilage.

Anesthesia

Rabbits were anesthetized by the intramuscular administration of 5 mg/kg xylazine hydrochloride and 35 mg/kg ketamine hydrochloride, supplemented by 0.1 ml ketamine hydrochloride administered intramuscularly as required. Cefuroxime 30 mg/kg was given to all rabbits intramuscularly.

Human amniotic fluid collection

HAF obtained from routine diagnostic amniocentesis performed in the second trimester and was provided by the prenatal diagnosis unit. Oral consent of the patients was received for each collection. HAF was stored at −80°C until use.

Surgical procedure

A midsternal skin incision was performed with the rabbits in a supine position. The pectoral muscles were carefully dissected and the costal cartilages exposed. Subperichondrial third and fifth costal cartilage resections were carried out by sharp dissection. Care was taken not to enter the perichondrium. The perichondrial bed was closed with continuous 3/0 polygactin sutures. Closure was done after the administration of 0.1 ml HAF into the perichondrial bed in group 2 rabbits and after the administration of 0.1 ml HAF into the perichondrial bed and the reimplantation of reshaped cartilages in group 3 rabbits (Fig. 1). The pectoral muscles were approximated with 2/0 polyglactin sutures, and the skin incision was closed with 2/0 polypropylene sutures.

Histopathological evaluation

Rabbits were sacrificed by the administration of high dose Xylazine (15 mg/kg) at two, eight or 12 weeks after surgery. Six rabbits from each of the three groups were sacrificed at either two, eight or 12 weeks after surgery. The sternum and bilateral costal cartilages were resected for specimens. Specimens were fixed in 10% formalin and embedded in paraffin for histological examina-
tion, and 4-mm thick sections were cut from the paraffin-embedded specimens. Sections were stained with hematoxylin and eosin for general examination and with Masson’s trichrome to assess scar tissue (Fig. 2). Neochondrogenesis in the perichondrial beds of the resected costal cartilages was evaluated. Light microscopy was used to determine neochondrogenesis, scar tissue, the diameter of neochondrogenesis, and calcification (Table 1). A numerical score, based on a modification of the histological score used to evaluate fracture healing, was used to assess neochondrogenesis and scar tissue formation [18] (Table 2). Ossification in the perichondrial bed is undesirable as it decreases the flexibility of the chest wall. In cases where we found ossification, we decreased the numerical score by one point (Fig. 3). The diameter of neochondrogenesis was measured in the light microscope by special technique and values between 90 and 180 µm were considered normal. These values were based on the diameters of unresected costal cartilages.

Statistical analysis
Data was analyzed using SPSS 16.0 for Windows. The statistical significance of numerical scores, the diameter of neochondrogenesis, and calcification was determined with the chi-square test. A value of *p* < 0.05 was considered significant.

Results

In order to determine the extent of neochondrogenesis in the perichondrial bed and the effect of HAF administration on neochondrogenesis, numerical scores and neochondrogenesis diameters were compared between the control group (group 1) and the group which received HAF (group 2). Numerical scores for the right perichondrial bed were significantly higher for group 2 rabbits compared to group 1 rabbits (*p* < 0.05). But there was no

**Table 1** Numerical scores and chondrogenesis diameters at the end of the 12th week.

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Animal no.</th>
<th>Right num. score</th>
<th>Chondrogenesis diameter on the right (µm)</th>
<th>Left num. score</th>
<th>Chondrogenesis diameter on the left (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>37</td>
<td>3</td>
<td>296</td>
<td>4</td>
<td>173</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>3</td>
<td>122</td>
<td>2</td>
<td>81</td>
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<td>90</td>
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<td>97</td>
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<td>135</td>
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<td>144</td>
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<td>1b</td>
<td>37</td>
<td>4</td>
<td>221</td>
<td>4</td>
<td>171</td>
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<td>4</td>
<td>198</td>
<td>3</td>
<td>122</td>
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<td>191</td>
<td>5</td>
<td>154</td>
</tr>
</tbody>
</table>

**Table 2** Numerical scoring scheme used for the histopathological evaluation of the perichondrial bed.

<table>
<thead>
<tr>
<th>Score</th>
<th>Histopathological findings in the perichondrial bed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fibrous tissue</td>
</tr>
<tr>
<td>2</td>
<td>Predominantly fibrous tissue with small amount of cartilage</td>
</tr>
<tr>
<td>3</td>
<td>Equal mixture of fibrous tissue and cartilaginous tissue</td>
</tr>
<tr>
<td>4</td>
<td>Predominantly cartilage with a small amount of fibrous tissue</td>
</tr>
<tr>
<td>5</td>
<td>Cartilage</td>
</tr>
</tbody>
</table>

**Fig. 2a to d** Numerical scores. 2 points (a), 3 points (b), 4 points (c), 5 points (d) (HE × 50).
significant difference in the numerical scores for the left peri-
chondrial bed between groups ($p > 0.05$). The diameter of chon-
drogenesis did not differ significantly between these groups ($p > 0.05$).

When we compared the control group (group 1) and the group
which had received HAF and undergone cartilage reimplantation
(group 3), the difference in numerical scores was not significant
at the end of second week for the left and right perichondrial
beds. But the difference was significant by the end of 12th week.
The diameter of chondrogenesis did not differ significantly be-
tween these groups ($p > 0.05$) (Table 3).

In order to determine the effect of cartilage reimplantation we
compared the group which received HAF (group 2) and the group
which received HAF and underwent cartilage reimplantation
(group 3). The difference was not significant with regard to nu-
umerical scores or diameters ($p > 0.05$). According to these findings
the reimplantation of reshaped cartilages obtained from the re-
moved cartilages into the perichondrial bed did not contribute
to neochondrogenesis.

Calcification was seen in the perichondrial bed at the end of 12th
week. There was a difference between groups 1 and 2; however,
this difference was not statistically significant.

We mentioned above them comprehensively. But we presented
them as left perichondrial bed (for partial resection) and right
perichondrial bed (for total resection).

**Discussion**

Whether the correction of pectus excavatum is necessary, which
operation should be performed, and the timing of the operation
are still controversially discussed in the literature. In the past,
some investigators suggested that PE should be repaired early in
life to allow the lungs to grow. However, the results of early oper-
ations were often disastrous because, after the radical resection
of costal cartilages, the immature cartilages were unable to sup-
port the repair and many patients developed restrictive thoracic
dystrophy [13]. The repair of PE after adolescence seems to be
more definitive and offers long lasting results.

Stabilization of the sternum has not been a problem in the last
decade as struts or bars have been placed behind, over, or
through the sternum or a titanium plaque has been placed over
the sternum to ensure stability [1, 19, 20]. Healing of the peri-
chondrial bed is very important to ensure that the chest wall is
not constricted. If fibrous tissue develops in the perichondrial
bed following cartilage resection, this will result in a restrictive
chest wall. Some investigators have proposed preserving the
growth centers at the costochondral junction to increase neo-
chondrogenesis [21, 22]. However, their studies were performed
in young animal models and did not consider the importance of
preserving growth centers at the costochondral junction in adult
animals. We used adult rabbits and did not find a difference be-
tween the group which received HAF and the control group after
partial resection. But the difference was statistically significant when we performed total resection, indicating that it is important to preserve the growth centers at the costochondral junction in adult animals. The correction of PE sometimes requires extensive cartilage resection in adults, and the growth centers at the costochondral junction are not enough to restore the perichondrial bed with cartilages. We suggested that HAF can promote cartilage formation in the perichondrial bed.

In the area of reconstructive plastic surgery, it has been demonstrated that perichondrial grafting, growth factors such as IGF, bFGF and EGF, and extracellular matrix proteins promote chondrocyte differentiation and proliferation. HAF obtained during the second trimester of gestation is known to be rich in these growth factors and in extracellular matrix precursors [23]. HAF additionally contains high concentrations of high molecular weight HA and HASA, which stimulate neochondrogenesis in the perichondrium [13, 14]. The experimental study by Ozgener et al. has suggested that a preoperative injection of HAF underneath the free perichondrial grafts promoted the proliferation and differentiation of chondrocytes [24].

We had three reasons for using HAF rather than rabbit amniotic fluid [24]. First, the growth factors, the extracellular matrix precursors, and the HA and HASA content of HAF are already known from previous studies. Second, it is easy to collect sufficient HAF for the study because amniocentesis is a common procedure performed for prenatal diagnosis at 16–20 weeks’ gestation in the Department of Obstetrics and Gynecology at Gulhane Military Medical Academy. Third, no cellular immune response against HAF was observed in a rabbit model similar to that of our experimental model [25].

Nuss and colleagues suggested that the ideal age for their PE correction procedure was before puberty, primarily because an elastic chest wall was necessary for success of the operation [6]. In adults, open procedures such as partially minimally invasive procedures (Erlangen technique) or our clinic’s procedure are procedures of the choice for PE management [4, 5, 19, 20]. Extensive resection of deformed cartilages can be accepted as a cartilage defect. The injection of HAF in the perichondrial bed reduces the formation of fibrous tissues and increases neochondrogenesis. There is no consensus in the literature on whether surgical repair improves pulmonary and cardiovascular function. Recently, a meta-analysis performed by Malek et al. indicated that surgical repair of PE significantly improves cardiovascular function [11]. But another meta-analysis showed no significant improvement in pulmonary function after surgical repair [8]. In some studies, an evaluation of lung volumes up to 3 years post-repair showed a decline of up to 10% from baseline values, presumably related to a reduction in chest wall compliance [26]. In our study, the group which received HAF demonstrated a significantly higher formation of new cartilage compared to the control group. If we could achieve healing of the perichondrial bed with chondrogenesis instead of fibrous tissue formation, chest wall compliance would not be reduced.

Croitoru et al. reported that scarification and ossification of the perichondrial bed was not detected on chest X-ray and was only visible on chest CT scan in 59% of patients previously operated for thoracic deformities [27]. In our study, ossification was detected in the perichondrial bed at the end of 12th week. There was a difference, although not statistically significant, between groups 1 and 2. The administration of HAF may have a preventive effect on ossification.

In conclusion, this experimental study suggests that an injection of HAF in the perichondrial bed of resected cartilages increases neochondrogenesis and decreases fibrous tissue formation. For human applications, further animal studies with commercially available HA preparations would be interesting, particularly with regard to breast wall reconstruction. Healing of the perichondrial bed and neochondrogenesis is very important for obtaining a flexible chest wall after correction of PE.

References


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