The role of muscle imbalance in the pathogenesis of shoulder contracture after neonatal brachial plexus palsy: a study in a rat model

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Background: An internal rotation contracture of the shoulder is common after neonatal brachial plexus injuries due to subscapularis shortening and atrophy. It has been explained by 2 theories: muscle denervation and muscle imbalance between the internal and external rotators of the shoulder. The goal of this study was to test the hypothesis that muscle imbalance alone could cause subscapularis changes and shoulder contracture.

Materials and methods: We performed selective neurectomy of the suprascapular nerve in 15 newborn rats to denervate only the supraspinatus and the infraspinatus muscles, leaving the subscapularis muscle intact. After 4 weeks, passive shoulder external rotation was measured and a 7.2-T magnetic resonance imaging scan of the shoulders was used to determine changes in the infraspinatus and subscapularis muscles. The subscapularis muscle was weighed to determine the degree of mass loss. An additional group of 10 newborn rats was evaluated to determine the sectional muscle fiber size and muscle area of fibrosis by use of images from type I collagen immunostaining.

Results: There was a significant decrease in passive shoulder external rotation, with a mean loss of 66%; in the thickness of the denervated infraspinatus, with a mean loss of 40%; and in the thickness and weight of the non-denervated subscapularis, with mean losses of 28% and 25%, respectively. No differences were found in subscapularis muscle fiber size and area of fibrosis between shoulders after suprascapular nerve injury.

Conclusions: Our study supports the theory that shoulder muscle imbalance is a cause of shoulder contracture in patients with neonatal brachial plexus palsy.
Shoulder impairment is the most common long-term complication and the major cause of morbidity after upper-trunk neonatal brachial plexus palsy (NBPP). A shoulder internal rotation contracture can develop in patients with incomplete recovery of the upper trunk. The constant position of internal rotation leads to early glenohumeral joint deformity, which is characterized by increasing glenoid retroversion and posterior humeral head subluxation (also known as glenohumeral dysplasia). The changes in bone and articular alignment have been extensively studied, whereas the pathogenesis of muscular changes has received little attention. The mechanism leading to subscapularis contracture has been postulated by 2 differing hypotheses: (1) denervation and (2) muscle imbalance. The denervation hypothesis states that denervation of the subscapularis muscle causes progressive muscle fibrosis, shortening, and contracture. This hypothesis has recently been supported by an experimental study in a mouse model.

Biopsy specimens from the subscapularis from children with NBPP do not show muscle fiber atrophy or fibrosis as predicted by the denervation hypothesis, despite loss of thickness in the muscle. Furthermore, several clinical and radiographic studies support the muscle imbalance hypothesis over the denervation hypothesis. The imbalance theory suggests that the subscapularis changes are due to incomplete or delayed external rotator muscle reinnervation. The weakened external rotators become overpowered by the internal rotators, leading to a persistent internal rotation posture. The lack of a passive stretch of the subscapularis leads to progressive shortening and muscle growth impairment.

The purpose of this study was to develop an animal model of muscle imbalance to test the hypothesis that muscle imbalance could lead to the internal rotation contracture seen in NBPP.

Materials and methods

This study was carried out following the National Institutes of Health guidelines for the use of laboratory animals and with the approval of the local ethics committee for experimental animal use. We used 30 newborn rats from 2 pregnant Sprague-Dawley Oncins France Strain A rats, 15 rats from each, in this study. The rat pups were randomly divided into 2 groups of 15: one group underwent an isolated neurectomy of the suprascapular nerve (suprascapular injury group), whereas the other group underwent an identical approach to the nerve without neurectomy (sham group). Only 1 upper limb from each pup was used, leaving the uninjured contralateral limbs as the native control group. Functional evaluation, magnetic resonance imaging (MRI), and subscapularis muscle weighing were performed 4 weeks after surgery, as described later.

An additional group of 10 newborn rats undergoing a neurectomy of the suprascapular nerve was evaluated at weekly intervals to measure passive shoulder external rotation and to determine the sectional muscle fiber size and muscle area of fibrosis by use of images from type I collagen immunostaining (histology group).

Newborn rat surgery

Five-day-old rat pups from the suprascapular injury group and the histology group underwent right brachial plexus surgery under general anesthesia with isoflurane. A surgical microscope was used for dissection. A transverse incision inferior to the clavicle was made with splitting of the pectoralis major and minor muscles to expose the brachial plexus. The suprascapular nerve was identified, and a neurectomy was performed with micro-scissors just distal to the branch point of the superior subscapular nerve. The skin incision was closed with running No. 7-0 polypropylene suture. Rat pups from the sham group underwent the same incision, exposure, and closure, but no neurectomy was performed.

Functional evaluation

Any gross gait abnormality was recorded just before the rats were killed. The animals were then killed with pentobarbital sodium injected intraperitoneally after sedation. Passive glenohumeral joint external rotation was measured immediately after the animals were killed. We used a modification of the method described by Nikolaou et al. Unlike the previously described method that measured external rotation in 90° of forward elevation of the shoulder, we performed our measurements with the rats positioned in full adduction with neutral flexion and extension of the shoulder. We performed our measurements with the rats positioned in full adduction with neutral flexion and extension of the shoulder. Our method replicates the way in which we measure external rotation clinically in children. The neutral position of the shoulder was defined as the shoulder in 0° of abduction and the elbow in 90° of flexion with the front limbs up ventrally perpendicular to the examination table. After scapular stabilization with the thumb, each shoulder was placed in maximal external glenohumeral joint rotation and photographed with a 12-megapixel digital reflex camera (Canon EOS 1100D; Canon, Tokyo, Japan) stabilized on a tripod perpendicular to the rat (Fig. 1).

The angle formed by the forearm and the animal’s midline was defined as glenohumeral external rotation. Measurements were performed digitally with Osiris software (Apple, Cupertino, CA, USA). The nonoperative side was measured and used as a control. A pilot study of 10 rats tested the reliability of this measurement method. Both limbs were positioned and photographed 3 times each, and each photograph was measured twice. The intraclass


Keywords: Neonatal brachial plexus palsy; glenohumeral dysplasia; shoulder contracture; subscapularis muscle; muscle imbalance; muscle growth
correlation coefficient was greater than 0.93, suggesting high reliability.

The rats from the histology group were evaluated for passive external rotation at weekly intervals for 4 weeks following the same measurement protocol. Measurements from the first week to the third week were performed with the rats anesthetized with an isoflurane-oxygen mixture, whereas the measurement at the fourth week was performed after the animal was killed.

**MRI evaluation**

After measurements of external rotation, the animals were placed supine with the front limbs on the abdomen into a 7.2-T MRI Biospect scanner (Bruker, Fällanden, Switzerland). Both shoulders were independently evaluated with RARE 1-mm axial TR 4000 T 30 sequences in the sagittal oblique and oblique axial planes parallel to the scapula. To standardize the cross-sectional slice chosen for measurements, the axial image selected to optimally visualize the subscapularis and infraspinatus muscles was just inferior to the spine of the scapula (Fig. 2). The point of maximum thickness was measured on the MRI scans of the involved and uninvolved shoulders. Measurements were made by use of Osirix software by a pediatric musculoskeletal radiologist who was blinded to specimen group. For reliability analysis, each measurement was repeated 3 times over 3 different days. All measurements were calculated by the mean of the 3 measures. The intraclass correlation coefficient was greater than 0.96, showing high reliability.

**Subscapularis muscle weighing**

After MRI, the right and left subscapularis muscles were extirpated and weighed. Through an anterior midline incision, both the pectoralis major and the clavicle were sectioned to gain access to the subscapularis fossae. The entire subscapularis was cleaned and dried of any surface moisture and was then placed on a high-precision analytic scale (AV114, Adventurer Pro; OHAUS, Nänikon, Switzerland).

**Analysis of subscapularis muscle fiber cross-sectional area and area of fibrosis**

The 10 rats from the histology group were killed 4 weeks after surgery with pentobarbital sodium injected intraperitoneally after sedation, and the subscapularis muscles from both sides were extirpated. Measurements were performed using the images
obtained from immunofluorescence of type I collagen–stained subscapularis muscle cuts. For homogeneity purposes, all images used for quantification were obtained from cuts of the same region (middle part of the muscle) and at the same area of the muscle (inferior area adjacent to upper subscapularis nerve).

Fresh, whole subscapularis muscles were embedded and suspended in OCT compound (Tissue-Tek; Sakura Finetek Europe B.V., Alphen aan den Rijn, The Netherlands) and immediately frozen in liquid nitrogen. The tissue blocks were stored at −80°C until used. Ten-micrometer serial frozen muscle transverse sections were cut at below −20°C with an HM550E microtome (Microm, Walldorf, Germany) and mounted on 0.1% (wt/vol) poly-L-lysine pretreated slides. Muscle sections were fixed in cold (−20°C) aceton for 5 minutes, rinsed twice with phosphate-buffered saline solution (PBS), and blocked with 10% goat serum in PBS for 20 minutes. Primary antibody against type I collagen (ab292; Abcam, Cambridge, UK) was diluted in PBS with 1% bovine serum albumin and incubated in a humid chamber for 16 hours at 4°C. Next, muscle sections were washed 3 times with PBS and incubated with Alexa Fluor 488 anti-rabbit secondary antibody (A-11008; Invitrogen, Carlsbad, CA, USA). We performed quantification of fibrosis in the subscapularis muscle cuts. For homogeneity purposes, all images obtained from immunofluorescence of type I collagen–stained subscapularis muscles were converted to binary format for pixel counts to determine the ratio of collagen to muscle area.

Statistical methods

Nonparametric analysis was performed for comparisons. The Wilcoxon test was used to check differences in paired comparisons (control limbs vs affected limbs). The Mann-Whitney U test was used to compare right shoulders in the suprascapular injury and sham groups. The level of significance was set at $P \leq .05$ for all statistical analyses.

Results

Functional evaluation and passive shoulder joint motion

The results are depicted in Table I. After recovery from surgery, all rats maintained a grossly normal gait. There was a statistically significant decrease in passive shoulder joint external rotation in the involved shoulder of the suprascapular injury group, with a mean loss of 66° (95% confidence interval [CI], 62°-69°) ($P < .01$) compared with the contralateral shoulder (Fig. 1). No statistically significant differences were observed between both shoulders in the sham group ($P = .175$) or between the uninvolved shoulder in the suprascapular injury group and the involved shoulder in the sham group ($P > .268$).

Passive external rotation assessed at weekly intervals in the histology group showed a statistically significant decrease in the involved shoulder joint, with a mean loss of 70° (95% CI, 67°-73°) ($P < .01$) in the first week after surgery, 77° (95% CI, 75°-80°) ($P < .01$) in the second week after surgery, 69° (95% CI, 67°-72°) ($P < .01$) in the third week after surgery, and 68° (95% CI, 65°-71°) ($P < .01$) in the fourth week after surgery (Fig. 3). Comparing the mean loss of passive shoulder external rotation between weeks, we found a statistically significant greater loss only in the second week.

MRI evaluation and muscle changes

In the involved shoulder of the suprascapular injury group, there was a statistically significant decrease in the muscle thickness of the infraspinatus and subscapularis muscles, with mean losses of 40.1% (95% CI, 34.71%-45.58%) ($P < .01$) and 28.3% (95% CI, 20%-32%) ($P < .01$), respectively (Fig. 2). There was a significantly greater percentage loss of muscle thickness in the infraspinatus compared with the ipsilateral subscapularis muscle. In the sham group, no statistically significant differences were found in the infraspinatus and subscapularis muscle thicknesses between shoulders ($P = .255$). There was also no difference between the uninvolved shoulder in the suprascapular injury group and either shoulder in the sham group ($P = .295$).

Muscle weight

In the suprascapular injury group, there was a statistically significant decrease in the weight of the involved subscapularis muscle compared with the nonoperative shoulder, with a mean loss of 24.7% (95% CI, 20.3%-29.2%) ($P < .01$) (Fig. 4). In the sham group, no statistically significant differences were found in the weight of the subscapularis muscle between shoulders ($P = .290$). There was also no difference between the uninvolved shoulder in the suprascapular injury group and either shoulder in the sham group ($P = .227$).

Cross-sectional subscapularis muscle fiber area and fibrosis

No statistically significant differences were found in the cross-sectional subscapularis muscle fiber area, measured in relative area units, between the left (control) and right (suprascapular neurectomy) subscapularis: 0.311 (95% CI,
0.308-0.314) and 0.286 (95% CI, 0.216-0.356), respectively (P > .25). No statistically significant differences were found in type I collagen deposition area, measured as the percentage of the total muscle section area, between the left and right subscapularis: 29.2% (95% CI, 39.2%-19.2%) and 30.7% (95% CI, 21.9%-38.9%), respectively (P > .24).

Discussion

The precise sequence of events leading to glenohumeral joint contracture after neonatal brachial plexus injuries remains controversial. The loss of subscapularis thickness is a consistent MRI finding in children with an internal rotation contracture. Changes in the subscapularis from denervation of the muscle have been implicated as the primary cause of shoulder contracture after NBPP.1,2,8,11 The subscapularis muscle receives segmental innervation from C5, C6, and C7. In upper-trunk lesions, the subscapularis remains partially innervated.12 However, studies have shown that even in upper-trunk lesions, the lower subscapularis atrophies along with the upper subscapularis despite maintenance of innervation.12-14 Although atrophy of a partially innervated muscle could lead to a contracture, the contracture could also be due to hypoplasia resulting from disuse. We have shown that without an antagonist to internal rotation, a fully innervated subscapularis still atrophies or fails to grow and leads to an internal rotation contracture as early as 1 week after suprascapular neurectomy.

We have therefore shown that loss of external rotation strength is sufficient to create the clinical picture seen in patients with upper-trunk NBPP. Our data do not exclude the possibility that partial subscapularis denervation may also contribute to contracture formation. In contrast to our results, other authors have shown that complete resection of the external rotators does not lead to internal rotation contractures.6 Their model introduces variables that are not seen in clinical practice. In our model, the supraspinatus and infraspinatus alone were denervated. In the model of Nikolaou et al,6 the infraspinatus and teres minor were resected, leaving a functional supraspinatus. Clearly, there are factors that lead to disparate results between both studies that we cannot yet identify. The extensive dissection and resultant scarring from excision of the muscles may play a role. The animal species in both models were different as well: we used a larger rat model, whereas Nikolaou et al used a mouse model.

In a similar rat model, Li et al12 created complete upper-trunk lesions that resulted in loss of passive external rotation. Their results confirm that denervation of the shoulder external rotators and partial denervation of the subscapularis in a rat model lead to internal rotation contracture.

Table I  Shoulder rotation, subscapularis thickness, infraspinatus thickness, and subscapularis weight

<table>
<thead>
<tr>
<th>Shoulder passive external rotation (°)</th>
<th>Subscapularis thickness (cm)</th>
<th>Infraspinatus thickness (cm)</th>
<th>Subscapularis weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Involved shoulder in SSI group [mean (SD)]</td>
<td>22 (6.41)</td>
<td>0.141 (0.03)</td>
<td>0.150 (0.03)</td>
</tr>
<tr>
<td>Uninvolved shoulder in SSI group [mean (SD)]</td>
<td>88 (2.41)</td>
<td>0.197 (0.03)</td>
<td>0.252 (0.04)</td>
</tr>
<tr>
<td>Involved shoulder in sham group [mean (SD)]</td>
<td>86 (4.28)</td>
<td>0.201 (0.04)</td>
<td>0.261 (0.04)</td>
</tr>
</tbody>
</table>

Statistical significance

- Wilcoxon test between involved and uninvolved shoulders in SSI group.
- Mann-Whitney U test between involved shoulders in SSI and sham groups.
contracts similar to those seen in patients. Our model specifically targets denervation of a subgroup of muscles innervated by the suprascapular nerve, with the goal of further pinpointing the causes of the contracture. Our results mirror those seen by Li et al but without partially denervating the subscapularis.

Clinically, children with internal rotation contractures show changes in subscapularis thickness similar to those seen in our model. Furthermore, biopsy specimens from the subscapularis muscle in children with NBPP do not show signs of denervation but do show loss of muscle thickness and an increase in muscle stiffness. Likewise, our histologic data show no fiber atrophy and no increase in muscle fibrosis, despite a decrease in subscapularis thickness of 28% and decrease in weight of 24%.

In our rat model, the loss of subscapularis thickness and weight, as well as the histologic findings of lack of fibrosis and preservation of muscle fiber cross-sectional area, is consistent with muscle hypoplasia. This phenomenon is in line with the theory of Einarsson et al that states that in the absence of subscapularis passive stretching from the external rotator muscles, the subscapularis muscle will fail to grow and will lose excursion.

The weaknesses of our study were that we did not measure subscapularis excursion or stiffness to determine whether there was a contracture of the muscle itself. Furthermore, the contribution of the capsule to internal rotation contracture was not assessed. However, the fact that the loss of passive external rotation occurred within the first week after nervectomy argues against a capsular origin for the contracture. Our data suggest that in this model, the contracture is first the result of muscular imbalance. Secondary capsular changes may occur later and contribute to overall shoulder stiffness.

The neonatal neural injury of our animal model, though not typically seen in clinical practice, was designed solely to evaluate the hypothesis that muscle imbalance alone is sufficient to cause internal rotation contractures. We have shown that muscle imbalance alone is sufficient to cause the type of contractures seen in clinical practice. Further studies are under way focusing on the alterations in the cellular and growth factor milieu from the loss of passive muscle elongation that lead to subscapularis hypoplasia.

Conclusions

Our study supports the theory that shoulder muscle imbalance with external rotation weakness is a cause of shoulder contracture in patients with NBPP. Macroscopic and microscopic analyses of the subscapularis show muscle changes similar to those found in children.

Disclaimer

This work was funded by Instituto de Salud Carlos III, grant FIS PI10/01357, cofinanced by the European Regional Development Fund, Fundació Privada A. Bosch, and Fundação Santa-Maria-Silva, for materials used in this study. The authors, their immediate families, and any research foundations with which they are affiliated have not received any financial payments or other benefits from any commercial entity related to the subject of this article.

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