A Model of Extraforaminal Brachial Plexus Injury in Neonatal Mice

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Introduction:
Brachial plexus birth palsy occurs in 1-3 per thousand live births, and causes permanent neurologic dysfunction in up to 30% of cases. The degree of injury and its recovery vary widely, with some infants recovering normal function and some developing debilitating secondary muscular and skeletal deformities. The pathophysiology of the effects of neonatal denervation on postnatal muscle development and function are not well understood, in large part to the absence of a suitable animal model that physiologically replicates the typically postganglionic injury in humans. The current study describes a model of extraforaminal brachial plexus injury in neonatal mice, with preliminary analysis of muscle immunohistochemistry.

Methods:
After obtaining approval from the Institutional Animal Care and Use Committee, unilateral extraforaminal brachial plexus injuries were surgically created in five mice at 5 days of age. The brachial plexus was exposed through a supraclavicular approach. After division of the omohyoid muscle, the C5 and C6 nerve roots were identified and transected. Following wound closure and recovery, the motor function of the limb was examined to confirm paralysis of appropriate muscle groups to simulate an upper trunk injury in humans.

Four weeks following surgery, motor function in both upper limbs was assessed by observation of gait, reaching, feeding, and grasping behaviors, and for each joint function, active muscle strength was recorded as absent, partial, or normal. Passive range of motion at the shoulder and elbow was then measured under sedation. The mice were then sacrificed and the biceps, triceps, and subscapularis muscles were harvested from both upper limbs for analysis. The range of motion of the elbow was measured immediately before and after biceps harvest. Muscle was post-fixed in 4% paraformaldehyde and quickly frozen in an acetone/dry ice slurry. Tissue was sectioned and subsequently stained with hematoxylin and eosin (H&E) for light microscopy or neurofilament antibodies and rhodamine-conjugated alpha-bungarotoxin for fluorescence microscopy. The combination of neurofilament-labeled axons and bungarotoxin-labeled neuromuscular junction allowed for an immunocytochemical examination of muscle innervation.

Results:
Following surgery, all 5 mice had clinical motor deficits mimicking upper-trunk injuries in humans: no active shoulder abduction (AB) or external rotation (ER), and no active elbow flexion (EF), but preservation of distal function (EE, WE, GR). One mouse died in the 3rd week post-operatively. The 4 remaining mice were examined at day 28 following operation. Two had full neurologic recovery, with normal active motion in all measured functions (AB, ER, EF, EE, WE, GR). The remaining two had no recovery of shoulder function (AB, ER) and partial recovery of elbow flexion (EF), but preservation of distal function (EF, WE, GR). Passive joint range of motion was measured at the shoulder (ER) and elbow (EF, EE). In the two mice with complete functional recovery, there were no detectable differences in joint range of motion between the operated and unoperated sides. In the two mice with persistent deficits, passive ER in adduction on the operated side was diminished by 20 and 40 degrees compared to the unoperated side, and passive EE was similarly limited by 25 and 30 degrees.

At muscle harvest, in the two mice with clinical recovery, there was no difference in gross appearance between the two sides in any muscle harvested. In the two mice without clinical recovery, there was severe atrophy of the biceps and deltoid. In the harvesting of the biceps muscle, the initial cut was performed at the musculotendinous junction proximal to the elbow joint. This cut, which left the joint capsule intact, allowed immediate correction of the restriction of passive elbow extension.

Microscopic analysis of biceps and triceps of the two severely affected mice confirmed the grossly observed atrophy. (Figure 1) Hematoxylin and eosin stain of denervated biceps revealed large clusters of atrophic fibers. The ipsilateral triceps appeared largely unaffected, confirming the specificity of the C5, C6 extraforaminal root lesion. Likewise, most if not all triceps acetylcholine receptors visualized with bungarotoxin were occupied by nerve fibers. Defined biceps neuromuscular junctions were difficult to find.

DISCUSSION:
The current study confirms the ability to create a brachial plexus lesion in neonatal mice clinically mimicking the typical upper trunk injury in humans. While others have developed models of intraforaminal injury in neonatal rats, the current model has three distinct advantages: 1. The postganglionic nature of the lesion more closely recreates the neurologic pathophysiology of the typical upper trunk lesion in humans, which is most often postganglionic in the C5 and C6 roots. 2. The extraforaminal location of the lesion allows more variability in healing potential, as seen in the current study, which could be characterized and/or modulated with refinement in the technique in future studies to ascertain pathophysiologic differences in muscle development between mild and severe injuries. 3. The use of mice rather than rats allows direct correlation with ongoing molecular genetic investigation of postnatal neuromuscular development.

Preliminary observations from application of this model in a small number of animals suggest that 1. joint contracture following neonatal brachial plexus injury correlates with degree of functional recovery, and 2: the limited passive range of motion is a primarily muscular rather than capsular problem. Further research with this model will refine the technique and investigate the molecular and cellular perturbations in postnatal skeletal muscle following neonatal denervation, with an eye toward developing rational rather than empirical interventions to prevent and treat secondary muscular deformities in children with brachial plexus birth palsy.

Figure 1: C5/C6 extraforaminal root lesion denervates rat biceps muscle. A. H&E stain of biceps muscle reveals small atrophic fibers and abundant connective tissue. B. Denervated biceps muscle stained for neurofilament and bungarotoxin revealed occasional nerve fibers (arrowhead). C. Neurofilament and bungarotoxin stain of triceps muscle revealed intact neurofilament (arrow) and bungarotoxin positive (arrowhead) neuromuscular junctions.