

Identifying and Pharmacologically Correcting the Molecular Pathophysiology of Contractures in Neonatal Brachial Plexus Injury

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Purpose: Neonatal brachial plexus injury (NBPI) causes disabling contractures that cannot be fully prevented or corrected, largely because their pathophysiology is incompletely understood. Research in a mouse model has shown that contractures result at least in part from impaired postnatal muscle growth, but the mechanism of this impaired muscle growth is unknown. The current work uses a mouse model to experimentally assess and correct muscle protein imbalance (synthesis versus degradation) as a mechanism of impaired muscle growth and contractures following NBPI.

Methods: Unilateral global (C5-T1) NBPIs were surgically created in 5-day-old mice, which permanently denervates the forelimb and reliably causes shoulder and elbow contractures 4 weeks post-NBPI. Protein synthesis was measured in denervated and contralateral control elbow flexor muscles within 4 weeks post-NBPI by incorporation of puromycin, a nucleoside analog, and by expression of major structural and contractile proteins by RNA-sequencing and Western blot. Protein degradation was similarly measured by K48-linkage specific polyubiquitin, an indicator of protein

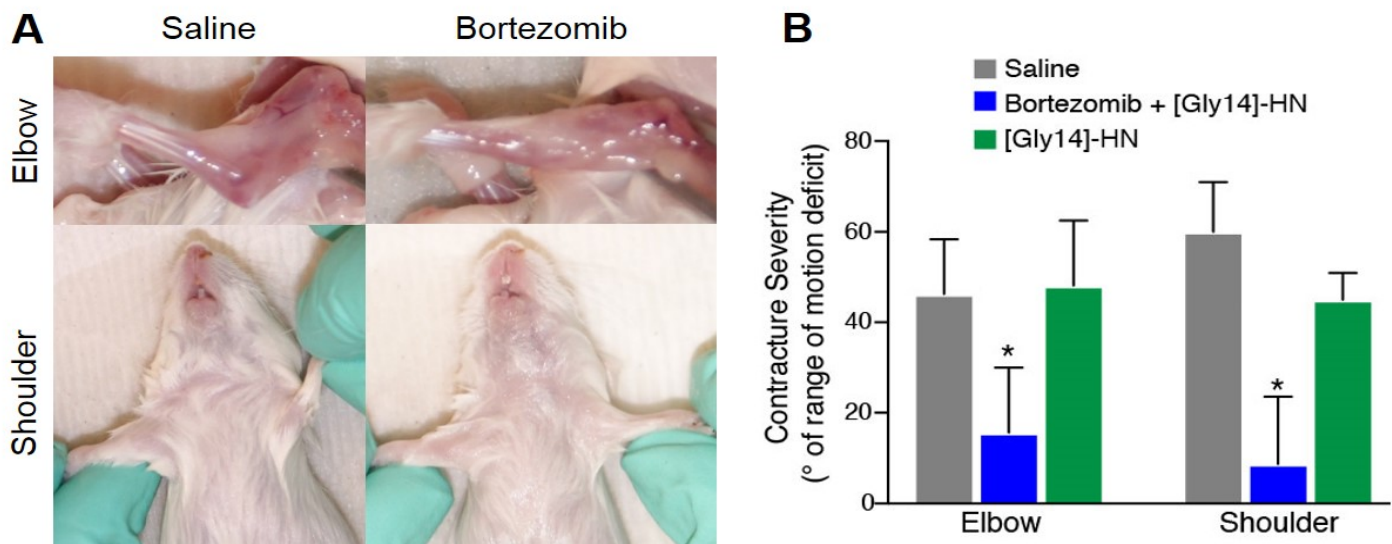


Figure 1: Prevention of contractures with bortezomib+[Gly14]-humanin. A) Representative images of elbow and shoulder contractures (left side denervated) in mice treated with saline and bortezomib. B) Contractures measured as difference in degrees of range of motion between denervated and contralateral control limbs, blinded to treatment group. [Gly14]-HN, given to limit bortezomib toxicity, had no effect alone. * $p < 0.001$

degradation by the proteasome pathway, and by expression of protein degradation markers by RNA-sequencing. Subsequently, to test the ability of proteasome inhibition to prevent contractures, bortezomib, a 20S proteasome inhibitor, was co-administrated with [Gly14]-Humanin G ([Gly14]-HN, to limit bortezomib toxicity) systemically for 4 weeks post-NBPI. Saline and [Gly14]-HN alone were administered separately as controls. Shoulder and elbow contractures were measured 4 weeks post-NBPI, and denervated and control elbow flexor muscles were assayed for 20S proteasome activity, volume and cross-sectional area (CSA) by microCT, and sarcomere length by DIC microscopy.

Results: NBPI did not reduce muscle protein synthesis, measured either by puromycin incorporation or by RNA and protein levels of all major structural and contractile proteins. However, NBPI increased protein degradation, as indicated by a doubling of K48-linkage specific polyubiquitin and increased expression of Trim63/MurF1, a driver of proteasome-mediated protein

degradation. Bortezomib + [Gly14]-HN effectively prevented contractures following NBPI compared to saline and [Gly14]-HN alone ($p<0.001$). Bortezomib blunted the denervation-induced increase in proteasome activity ($p<0.001$), and rescued muscle growth in volume ($p<0.0001$), CSA ($p<0.001$), and sarcomere length ($p=0.03$).

Conclusion: Contractures following NBPI are associated with increased muscle protein degradation counteracting normal protein synthesis. Inhibition of the proteasome pathway of protein degradation improves growth of denervated muscle and prevents contractures following NBPI.

Significance: This study identifies a pathophysiologic mechanism of impaired muscle growth and contracture formation following neonatal brachial plexus injury and demonstrates the first ever successful pharmacologic strategy to prevent these contractures by targeting a causative molecular mechanism.