The Efficacy of Proteasome Inhibitors in Pediatric Muscle Contractures in Neonatal Brachial Plexus Injury (NBPI)

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Background

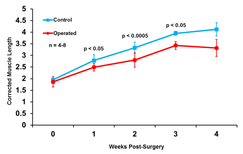
Neonatal Brachial Plexus Injury (NBPI)

- Neonatal Brachial Plexus Injury (NBPI) is the most common birth injury
- Leads to upper limb paralysis and the secondary formation of muscle contractures
- Contractures are defined as tightness or stiffness in the joints
- Current treatments are ineffective in restoring joint function and mobility

Contracture Pathogenesis

- Contractures following NBPI result from impaired longitudinal growth of the denervated muscle.[1]
- The deficit in muscle length is driving by elevated levels of proteasome-mediated protein degradation (Figure 1A).[2]

A



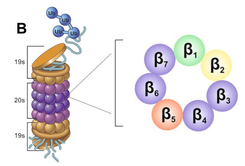
We must therefore investigate the role of the proteasome in contracture formation.

Proteasome Inhibition

- Proteasomes are protein complexes that degrade/break down proteins by proteolysis (Figure 1B).
- The 26S proteasome contains a 20S core catalytic site where proteins are broken down.
- There are three active subunits in the 20S core: beta one, two, and five.[3]
- These subunits pharmacologically targeted by proteasome inhibitors to block proteasome activity (Figure 1C).

In this current study, we investigate the role of the proteasome in contracture development, specifically the efficacy and therapeutic windows of the different classes of proteasome inhibitors. We speculate that the 20S proteasome is a key regulator in contracture formation. (Figure 1B).

B



C

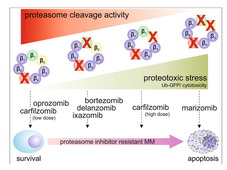
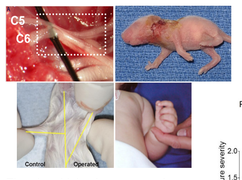


Figure 1: Overview of neonatal brachial plexus injury. A) Conceptual representation of the protein balance in growing and denervated muscle. **B)** Figure of the 26S proteasome with the beta subunits in the 20S core catalytic site. **C)** Figure of the different classes of proteasome inhibitors that have been approved for treatment in multiple myeloma(MM).

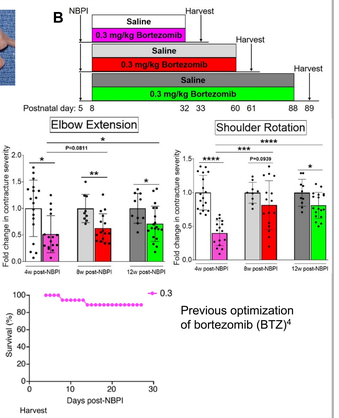
Methods

A



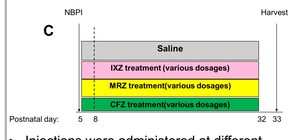
- The brachial plexus complex was surgically excised in 5-day old CD1 mice (top left panel), resulting in forelimb paralysis (top right panel).[1]
- Following NBPI surgery, the mice developed physiologically relevant contractures (limited shoulder rotation and elbow hyperflexion) by 4 weeks post-NBPI (bottom panels).

B



Previous optimization of bortezomib (BTZ)[4]

C



- Injections were administered at different iterations depending on the starting point of (P5) or (P8) every other day till 4 weeks post-NBPI (P32).
- Assessment of range of motion(ROM) – shoulder rotation and elbow extension
- Survival curve to monitor toxicity of proteasome inhibitors(PI)

Figure 2: Mouse model of NBPI, prior findings with bortezomib, and experimental design. A) Mouse model of unilateral NBPI. **B)** Bortezomib (BTZ), a proteasome inhibitor, optimally prevent elbow and shoulder contractures at 4 weeks post-NBPI with low mortality. Contracture severity is calculated as the difference in passive elbow extension and shoulder rotation between the denervated (NBPI) side and the contralateral control side, and then, the mean contracture severity among saline treated animals is set to 1.0 to allow normalization of the treatment groups across time points (n = 9-19 mice). All data are presented as mean ± s.d. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. **C)** Schematic of the experimental process to follow with newer generation of proteasome inhibitors – Ixazomib (IXZ), Marizomib (MRZ), and Carfilzomib (CFZ).

Proteasome Inhibition using MRZ

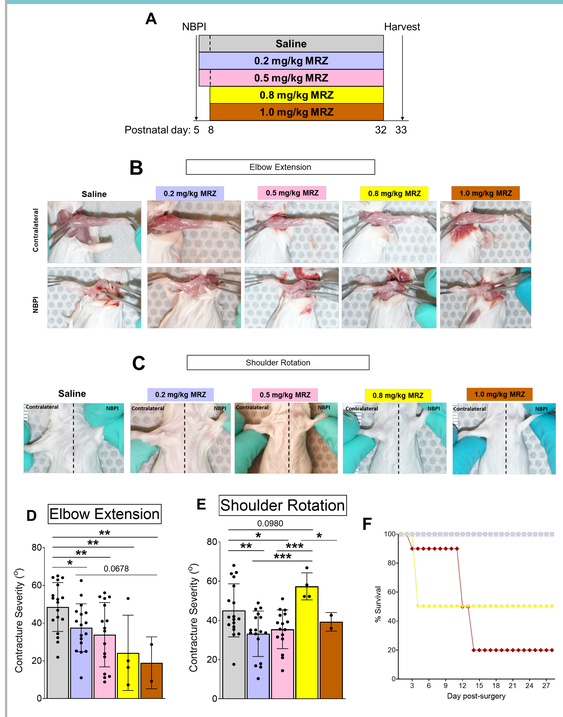


Figure 3: Contracture prevention using marizomib treatment is mitigated by the toxic effects of the drug. A) Schematic depicting the timeline of treatment in the experiment. **B&C)** Representative images of denervated (NBPI) and contralateral forelimbs and quantitative analyses of **D)** elbow extension and **E)** shoulder rotation. **F)** Survival Curve shows increased dose concentration of MRZ led to a lower mortality rate. Contracture severity is calculated as the difference in passive elbow extension or shoulder rotation between the denervated (NBPI) side and the contralateral (control) side. All data are presented as mean ± s.d. *P<0.05, **P<0.01, ***P<.001.

Proteasome Inhibition using IXZ

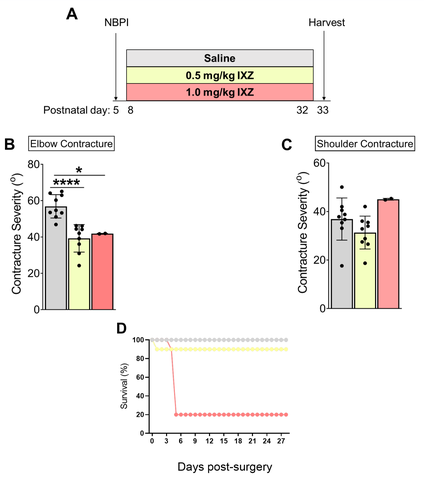


Figure 4: Ixazomib treatment is limited by its toxic effects with increased dosage concentration. **A)** Schematic depicting the timeline of IXZ treatment. **B)** Quantitative analyses of elbow extension and **C)** shoulder rotation. **D)** Survival Curve shows increased dose concentration of IXZ led to a lower mortality rate. Contracture severity is calculated as the difference in passive elbow extension or shoulder rotation between the denervated (NBPI) side and the contralateral (control) side. All data are presented as mean ± s.d. *P<0.05, ****P<0.0001.

Proteasome Inhibition using CFZ

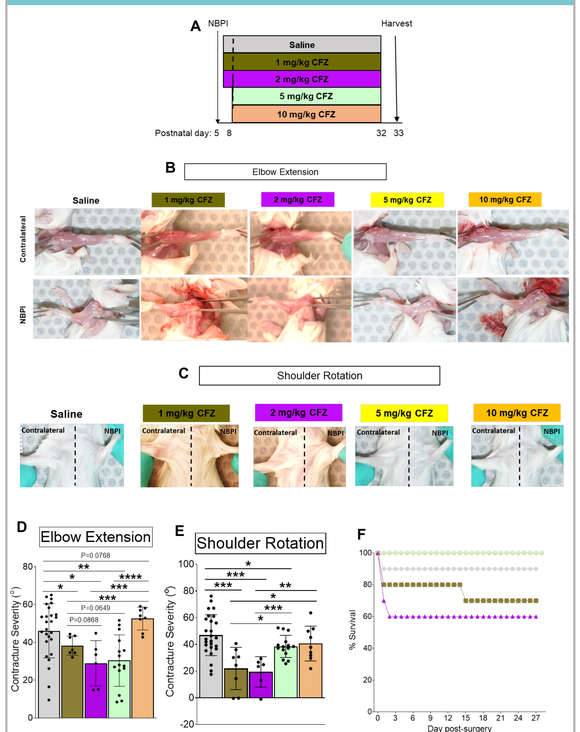


Figure 5: The efficacy of carfilzomib is limited by its therapeutic window. A) Schematic depicting the timeline of CFZ treatment. **B&C)** Representative images of denervated (NBPI) and contralateral forelimbs and quantitative analyses of **D)** elbow extension and **E)** shoulder rotation. **F)** Survival curve showing mortality rates from saline treatment and the different doses of CFZ. . Contracture severity is calculated as the difference in passive elbow extension or shoulder rotation between the denervated (NBPI) side and the contralateral (control) side. All data are presented as mean ± s.d. *P<0.05, **P<0.01, ***P<.001, ****P<0.0001 .

Summary of Proteasome Inhibition Data

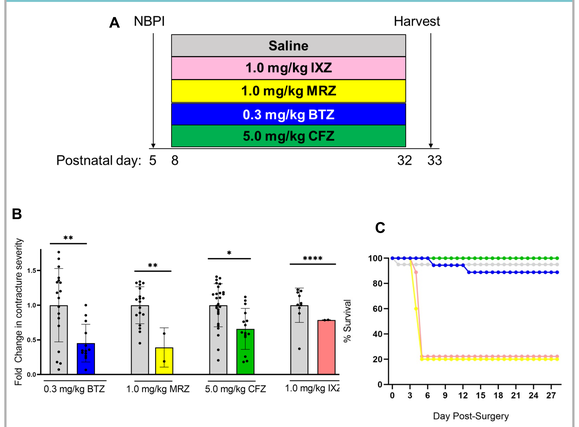


Figure 6: Summary data of all four proteasome inhibitors. **A)** Summary schematic depicting the most optimal dosage for each proteasome inhibitor that was utilized to determine the optimal method of treatment. In **B)** contracture severity is calculated as the difference in passive elbow extension and shoulder rotation between the denervated (NBPI) side and the contralateral control side, and then, the mean contracture severity among saline treated animals is set to 1.0 to allow normalization of the treatment groups across time points (n = 9-19 mice). **C)** Survival curve compiling the most optimal dosages of each proteasome inhibitor, highlighting the limitations of MRZ and IXZ treatment due to toxicity. Statistical analyses: (B) unpaired two-tailed Student's t tests. *P<.05, **P<.01, ****P<.0001. All samples represent independent biological data points.

Conclusions/Future Directions

A

Therapeutic Window

Proteasome Inhibitors	Efficacy?	Toxicity?
Carfilzomib (CFZ)	↑	—
Marizomib (MRZ)	↑ ↑	↓ ↓ ↓
Ixazomib (IXZ)	↑	↓ ↓ ↓
Bortezomib (BTZ)	↑ ↑	↓

Conclusions

- As all classes of proteasome inhibitors reduce contracture formation, our results offer proof of concept for the proteasome as a key regulator of NBPI-induced contractures
- The narrow dose ranges of efficacy highlight the need for precise proteasome regulation in contracture prevention.

Future Directions

- The substantial toxicity of systemic proteasome inhibition underscores the need to identify muscle-specific targets for suppressing protein degradation to prevent contractures.

Figure 7: Summary of findings. A) Table depicting the efficacy (therapeutic window) and toxicity of the different classes of proteasome inhibitors.

Acknowledgements

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4) Goh, Qingnian, et al. "Timing of Proteasome Inhibition as a Pharmacologic Strategy for Prevention of Muscle Contractures in Neonatal Brachial Plexus Injury." *The FASEB Journal*, 35(2):e21214.