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Intramuscular injection of collagenase clostridium histolyticum may decrease spastic muscle contracture for children with cerebral palsy

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A B S T R A C T

In cerebral palsy (CP), the spastic motor type is most common, associated with a velocity-dependent increase in muscle stiffness that precedes the development of fixed muscle contracture – a permanent shortening of the muscle tendon unit even when relaxed. Intra-muscular injections of botulinum toxin type A (BTX-A) have become popular for the treatment of spastic muscle contractures but unfortunately its use has not resulted in long-term functional benefits and, paradoxically, has been associated with a persistent loss of contractile material. Recent biomechanical work has shown that the stiffness of the CP muscle increases in proportion to total collagen content within the perimysial extra-cellular matrix. Thus, rather than the use of tone-reducing agents, we hypothesize that the focal use of a selective collagenase, injected into spastic muscle at an appropriate dilution and concentration, may serve to reduce the extent of muscle contracture, improving clinical range of motion and perhaps sarcomere length.

Introduction

Cerebral palsy (CP) occurs secondary to an insult to the developing brain, with musculoskeletal aspects that are progressive with growth [1]. In CP, the spastic type is most common, associated with a velocity-dependent increase in muscle stiffness that precedes the development of fixed muscle contracture – a permanent shortening of the muscle tendon unit (MTU) even when relaxed [2,3]. For young children, performing surgical lengthenings to treat these "short muscles" carries a higher risk of contracture recurrence. In this age group, non-surgical interventions, such as intra-muscular injections of Botulinum toxin type A (BTX-A) have become popular, focally decreasing spasticity by blocking the activity of the neuromuscular junction. Although this simple treatment reliably improves joint range of motion in the short term, this effect is transient and has not led to demonstrable long-term benefits [4]. In addition, BTX-A injection causes prolonged decreases in muscle strength and contractile material, with viable muscle being replaced with fibrofatty tissue [5].

The search for a safe and effective non-operative means to treat muscle contractures in CP is far from over. To help understand the mechanical and pathoanatomical properties underlying the etiology of CP muscle stiffness, our research group investigated the passive stress generated (i.e. stiffness) in myofibrils for children with CP as compared to a control group [6,7]. Muscle myofibrils are comprised of serially arranged sarcomeres, each containing overlapping actin and myosin filaments which slide over one another during muscle contraction. Our research showed that, rather than being stiff, the CP myofibrils themselves were instead highly elastic, with Young's elastic modulus approximately 40% less than for control tissue. As corroborated by other studies, we found *in vivo* sarcomere length to be substantially long (approximately 3.5 μm) [8], operating half-way down on the descending limb of the muscle force-length curve, with an expected decrease in actin-myosin overlap and thus force generating capacity.[9] Despite their decreased stiffness, CP myofibrils were found to be under increased passive stress at *in vivo* sarcomere lengths [6,7], perhaps contributing to their "overstretching".

Other studies have investigated the pathophysiology of CP muscle stiffness at higher structural levels. In a study involving biopsies of hamstring muscles in children with CP, Smith and colleagues showed that increased stiffness from within muscle fibre bundles was associated with an increase in collagen content (mostly Type I) of the extracellular matrix (ECM), rather than from the muscle fibers themselves [8]. This alteration in ECM was hypothesized to be in response to the increased passive stress imparted to the muscle at *in vivo* sarcomere lengths. These results were corroborated by other authors [10,11].

Recent biomechanical work suggested that a substantial amount of muscle passive load is borne by the ECM and that the stiffness of the entire muscle belly increases in proportion to total collagen content [12,13]. Given that collagen is the primary constituent of the ECM, the targeted use of intramuscular collagenase may provide the means by

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which more normal MTU length (and thus excursion) could be restored, without the need for invasive surgery, while at the same time preserving – and perhaps enhancing – the contractile abilities of the muscle tissue.

Hypothesis

With respect to the development of muscle contractures in CP, given the proposed contributions of increased passive stresses at *in vivo* (overstretched) sarcomere lengths and associated increases in ECM collagen content, we hypothesize that the focal use of a selective collagenase, injected into spastic muscle at an appropriate dilution and concentration, may serve to reduce the extent of muscle contracture, improving clinical range of motion and perhaps sarcomere length.

Evaluation of the hypothesis

Collagenases derived from enzymatic action of bacteria within the Clostridia species have been known to lyse the collagen framework of muscle tissue for almost a century, most effectively by Collagenase Clostridium histolyticum (CCH) [14]. CCH has subsequently been used successfully in the treatment of the collagen rich fibrous cords of both Dupuytren's contracture and Peyronie's disease. As a result of its favourable safety and effectiveness profile, the use of CCH for the treatment of these disorders led to its approval for use by the U.S. Food and Drug Administration (marketed under the name Xiaflex®, Endo Pharmaceuticals Inc. Malvern, PA, USA) [15]. CCH destroys and downregulates the Type I and III collagen while preserving Type IV collagen, the primary structural component in arteries, nerves, and veins [16]. In addition, although Type I and III collagens have been identified within both the endomysium and perimysium, the sarcolemma (the cell membrane surrounding individual muscle fibers) is composed primarily of Type IV and V collagens (and laminin), both relatively unaffected by CCH [17].

For this potential treatment to be successful, CCH should readily diffuse throughout the muscle belly, a very different scenario compared to its use in superficially located fibrous cords. The diffusion characteristics of CCH within skeletal muscle are currently unknown but a recent study investigating its successful use in uterine fibroids (benign tumours composed of smooth muscle and excessive ECM) suggested that the diffusion of CCH is substantial and related to drug concentration and dilution volume [18].

It is acknowledged that increased collagen content is not the only determinant of muscle stiffness in CP. Decreased sarcomerogenesis, increased sarcomere lengths, and adaptations involving the molecular spring protein, titin, may all have roles to play. Despite this, given the discussion above, we believe that the ECM likely plays a large enough role to warrant investigation of the treatment effects of CCH on CP muscle.

Discussion

The use of CCH to reduce collagen content in spastic muscle is expected to decrease muscle stiffness, improve joint range of motion, and possibly improve muscle strength. Initially, the extent of CCH diffusion throughout the muscle belly would need to be determined, dependent on concentration and volume. As has been previously reported for fibroids, methylene blue in conjunction with CCH could be used to determine the diffusion characteristics of the collagenase [18].

To test our major hypothesis, a randomized-controlled study, using mature hereditary spastic mice (B6c3a/a spa/+ mice), would be developed, wherein the hamstrings are injected with CCH in the experimental group (saline for controls). Two other comparator groups are envisioned: BTX-A alone and BTX-A plus CCH. This would ensure that the intervention is compared against both controls and the current 'gold standard'. The hereditary spastic mouse model has been used previously for the initial experiments investigating the effects of BTX-A on spastic muscle [19]. Akin to the protocol reported for Dupuytren's contracture, the mice would undergo a post-injection dynamic stretching program for one month [15]. Following this, a functional assessment using validated outcome measures would be applied and knee joint range of motion measured. The animals would then be sacrificed and MTU lengths measured. Sarcomere lengths would be determined by laser diffraction methods with the knee and hip joints at a standardized position during biopsy [8]. The tissues would undergo microscopic examination of global morphology and stained for collagen content and laminin (to confirm sarcolemma integrity).

It is anticipated that the treatment group will require stratification by CCH dose to determine the dose-response relationship.

If successful, the use of CCH for spastic muscle contractures could result in a relative decrease in passive stress imparted to the muscle, potentially resulting in an increase in joint range and a decrease in sarcomere length [10]. This shortening in sarcomere length is expected to be accompanied by an improvement in actin-myosin filament overlap with a corresponding improvement in muscle strength, a clinical manifestation adversely affected in CP [20].

It is further expected that laminin content and structural arrangement will be unchanged, suggesting preservation of the sarcolemma. Preservation of the smooth muscle cell membranes following CCH injection into uterine fibroids supports this view [18].

If our hypothesis is proven correct, the use of CCH for the treatment of spasticity-related contractures could represent a paradigm shift by which children with CP are spared surgical interventions in favour of a non-operative approach that promotes muscle lengthening without adversely affecting its contractile elements.

Author contributions

Dr. Jason Howard developed the core hypothesis and wrote the initial manuscript.

Dr. James Huntley critically reviewed the manuscript and suggested revisions which were incorporated.

Dr. H. Kerr Graham critically reviewed the manuscript and suggested revisions which were incorporated.

Dr. Walter Herzog critically reviewed the manuscript and suggested revisions which were incorporated.

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Conflict of interest statement

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