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Simulation of effects of botulinum toxin on muscular mechanics in time course of treatment based on adverse extracellular matrix adaptations

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ABSTRACT

BTX effects on muscular mechanics are highly important, but their mechanism and variability in due treatment course is not well understood. Recent modeling shows that partial muscle paralysis per se causes restricted sarcomere shortening due to muscle fiber–extracellular matrix (ECM) mechanical interactions. This leads to two notable acute-BTX effects compared to pre-BTX treatment condition: (1) enhanced potential of active force production of the non-paralyzed muscle parts, and (2) decreased muscle length range of force exertion (ℓ_{range}). Recent experiments also indicate increased ECM stiffness of BTX treated muscle. Hence, altered muscle fiber–ECM interactions and BTX effects are plausible in due treatment course. Using finite element modeling, the aim was to test the following hypotheses: acute-BTX treatment effects elevate with increased ECM stiffness in the long-term, and are also persistent post-BTX treatment. Model results confirm these hypotheses and show that restricted sarcomere shortening effect becomes more pronounced in the long-term and is persistent or reversed (for longer muscle lengths) post-BTX treatment. Consequently, force production capacity of activated sarcomeres gets further enhanced in the long-term. Remarkably, such enhanced capacity becomes permanent for the entire muscle post-treatment. Shift of muscle optimum length to a shorter length is more pronounced in the long-term, some of which remains permanent post-treatment. Compared to Pre-BTX treatment, a narrower ℓ_{range} (20.3%, 27.1% and 3.4%, acute, long-term and post-BTX treatment, respectively) is a consistent finding. We conclude that ECM adaptations can affect muscular mechanics adversely both during spasticity management and post-BTX treatment. Therefore, this issue deserves major future attention.

1. Introduction

Local injections of Botulinum toxin type-A (BTX) are commonly used to reduce spasticity by causing partial muscle paralysis and blocking the hyper-excitable stretch reflexes (Filippi et al., 1993; Giladi, 1997; Metezeau and Desban, 1982). However, BTX also affects muscle force substantially (Burgen et al., 1949; Longino et al., 2005; Yucesoy et al., 2012). As muscle is the motor for movement, BTX effects on muscular mechanics are functionally extremely important, but the mechanism of these effects and how they change in due treatment course is not well understood. Moreover, no projection is made about muscle function post-BTX treatment.

Finite element modeling is a powerful tool to assess such mechanisms. Recent modeling effects of BTX on muscular mechanics indicates that a vast majority of sarcomeres attain higher lengths compared to identical sarcomeres in pre-BTX treated muscle (Turkoglu et al., 2014). Such *"longer sarcomere effect"* (LSE), is characteristic to partial muscle paralysis per-se, ascribed to muscle fiber–extracellular matrix (ECM) interactions (Yucesoy, 2010). Modeling indicates that LSE can lead to an enhanced potential of active force production of the non-paralyzed muscle parts and a decreased length range of muscle force exertion (ℓ_{range}) as acute-BTX treatment effects. Therefore, BTX effects on muscular mechanics may not be limited to only a force reduction. Recent animal experiments confirm that and additionally show an increased passive muscle force (Ates and Yucesoy, 2014; Yucesoy et al., 2012, 2015). This is ascribed to an enhanced collagen content of the ECM, several days post-injection (Ates and Yucesoy, 2014). These findings indicate that longer-term exposure to BTX leads to muscle tissue adaptation and altered mechanical properties of the ECM. Moreover, although no direct information is available on the ECM properties and structure post-BTX treatment, persistence (Boyd and Graham, 1999) and even increase of contracture (Kay et al., 2004) have been reported in BTX treated patients. This suggests changes to ECM properties are sustained post-BTX treatment.

Taking these issues into account and using finite element modeling we aimed at simulating the time course of effects of BTX on muscular mechanics based on LSE and ECM adaptations. Specifically, we tested

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the following hypotheses: due to increased ECM stiffness, acute-BTX treatment effects (1) elevate in the long-term, (2) and are persistent post-BTX treatment.

2. Methods

2.1. Modeled BTX cases

To assess the mechanism of effects of BTX in the time course of treatment, four cases were modeled: (1) *Pre-BTX treatment* represents BTX-free muscle. Therefore, the entire muscle is fully activated. (2) *Acute-BTX treatment* represents effects of BTX immediately after muscle paralysis settles down. Partial muscle paralysis per se was modeled by leaving the middle half of the model inactive (*paralyzed parts*) and fully activating the remaining half (*activated parts*). (3) *Long-term-BTX treatment* models ECM adaptations due to BTX. Therefore, constitutive equations of elements modeling the ECM are manipulated for stiffer mechanical properties (see the subsequent Section). (4) *Post-BTX treatment* simulates mechanics of BTX treated muscle after partial muscle paralysis ceases. Adapted ECM properties are maintained, but the entire muscle is fully activated.

2.2. Description of the reference muscle model

Using a two-domain approach, a 3D-finite element muscle model (linked fiber-matrix mesh model: LFMM model) was developed (Yucesoy et al., 2002). This model consists of two meshes, occupying the same space, that are linked elastically. These meshes represent the ECM domain (matrix mesh) and intracellular domain (fiber mesh). Such modeling allows assessment of effects of muscle fiber–ECM interactions.

The two meshes are built using the self-programmed "myofiber" and "ECM" elements that were introduced as user defined elements into a finite-element analysis software (ANSYS Academic Teaching Advanced v.12.0 ANSYS. Inc. Canonsburg, PA, USA). The elements have eight nodes and linear interpolation functions. The tissues are considered as a continuum and a large deformation analysis is employed. A 3D local coordinate system representing the fiber, cross-fiber, and thickness directions is used.

For the myofiber element, the total stress acting only in the fiber direction equals the sum of the active stress of the contractile elements (representing active force exertion based on overlap of actin and myosin myofilaments) and the stress due to intracellular passive tension (originating from the intra-sarcomeric cytoskeleton, with a dominant role played by titin). It is assumed that the sarcomeres arranged in-series within muscle fibers have identical material properties.

The ECM element incorporates a two-component strain energy density function one of which accounts for the non-linear and anisotropic material properties (Fig. 1) and the other considers constancy of muscle volume.

The former component is described as follows:

$$W_1 = W_1(\epsilon_{ij}) \tag{1}$$

where

$$W_1(\epsilon_{ij}) = k.(e^{a_{ij}.\epsilon_{ij}} - a_{ij}.\epsilon_{ij}) \text{ for } \epsilon_{ij} > 0 \text{ or} \tag{2}$$

$$W_1(\epsilon_{ij}) = -W_1(|\epsilon_{ij}|) \text{ for } \epsilon_{ij} < 0 \text{ and } i \neq j$$

ϵ_{ij} are the Green–Lagrange strains in the local coordinates. The indices i and $j = 1...3$ represent the local cross-fiber, fiber and thickness directions respectively. k is initial passive stiffness constant and a_{ij} are stiffness coefficients.

For full details including description of remainder constitutive relationships, model parameters and activation procedure; see a review by Yucesoy and Huijing (2012).

One muscle element (a linked system of ECM and myofiber elements) represents a segment of a bundle of muscle fibers, its connective tissues and the links between them. Both matrix and fiber meshes are rigidly connected to single layers of standard hyperelastic elements (HYPER58) elements forming the muscles' proximal and distal aponeuroses. Standard spring elements (COMBIN39) are used to represent the transmembranous attachments i.e., the elastic links between the two meshes. This is a 2-node spring element, set to be uni-axial and having linear high stiffness representing non-pathological connections between the muscle fibers and the ECM (for an analysis of the effects of stiff or compliant links, see Yucesoy et al., 2002). Initially, these links have zero length.

The extensor digitorum longus (EDL) muscle of the rat was modeled. This muscle is unipennate with a minimal variation of its muscle fiber directions. The geometry of the model (Fig. 2a) is defined as the contour of a mid-longitudinal slice of the isolated EDL muscle belly. A whole fascicle is constructed by putting three muscle elements in series. Sixteen of those are arranged in parallel, filling the slice space. A combination of nodes along one side of a fascicle is referred to as a *fascicle interface*. Partial muscle paralysis modeled within slice space is shown in Fig. 2b.

2.3. Starting conditions employed in pre- and Acute-BTX treatment cases

(i) *Reference*: Muscle tissue properties are described above.

(ii) *m. Spastic-I*: Spastic muscle tissue was reported to be stiffer than normal (e.g., Sinkjaer and Magnussen, 1994; Tardieu et al., 1982). This is implemented by increasing stiffness of the ECM element (see Supplement I for full details).

(iii) *m. Spastic-II*: Another possible effect is deranged sarcomere force-length properties (Olesen et al., 2014; Ponten et al., 2007). Additional to increased ECM stiffness identically to m. Spastic-I, this is represented by changing properties of the myofiber element by imposing a shift of active stress–strain curve to negative fiber strains (see Supplement I for full details).

2.4. Adapted ECM properties employed in Long-term- and Post-BTX treatment cases

Recent experimental testing of rat EDL muscle exposed to BTX indicates increased amplitudes of muscle passive force (up to several folds) with shift of non-zero passive force exertion to shorter muscle lengths (Ates and Yucesoy, 2014). ECM element material constants are manipulated to incorporate such *adapted ECM* mechanical properties. For reference condition, k of adapted ECM is tripled, whereas a_{ij} are left unchanged if $i = j$ or reduced (by 40%), otherwise. The former allows enhanced normal stiffness also at negative strain and the latter avoids a change in shear stiffness since no information is available for such ECM adaptation. The resulting stress–strain curves are shown in Fig. 1. Also for m. Spastic-I and II conditions, ECM material constants are manipulated to achieve an equivalent adapted ECM.

2.5. Solution procedure

At the initial ℓ_m (28.7 mm), and in the passive state, the serial sarcomeres within muscle fibers were assumed to be in the undeformed state (i.e., strain equals zero, and sarcomere length approximates 2.5 μm). Local fiber strain, as a measure of change of length, reflects lengthening (positive strain) or shortening (negative strain) of sarcomeres.

Static analysis was performed, using a force-based convergence criterion (tolerance = 0.5%). Muscle origin was fixed whereas, after activation, the position of the insertion was changed to new isometric lengths.

2.6. Processing of data

ℓ–F_{curves} were studied to quantify passive and active force changes. All forces are normalized for the maximal total force within the data set.

The mean nodal fiber direction strain per fascicle interface is considered to represent *sarcomere length changes* and hence a metric for LSE. ℓ_m range studied includes lengths between a short (ℓ_m = 25.2 mm) and a long length (ℓ_m = 30.7 mm). Mean nodal fiber direction strains are assessed at those lengths.

Muscle optimum length, ℓ_{mo} is determined as the difference of passive and total muscle force. Shifts in ℓ_{mo} of Acute-BTX, Long-term BTX and Post-BTX treatment cases are compared to that of Pre-BTX treatment case within the range of muscle lengths studied. Additionally, muscle active slack lengths of each case are determined and their ℓ_{range} is calculated as the difference of that and ℓ_{mo}.

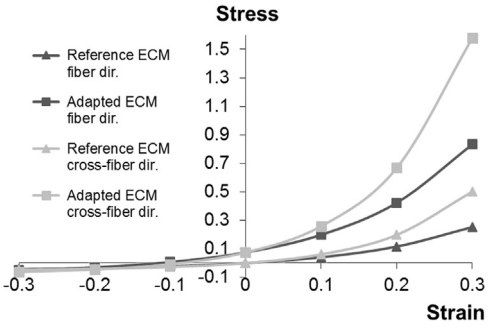

Fig. 1. Non-linear and anisotropic material properties of the extracellular matrix element in the local coordinates. The stress–strain characteristics of the element are shown for the fiber and cross-fiber directions. Material properties of *reference ECM* represents those of BTX free muscle (Huyghe et al., 1991). Material properties of *adapted ECM* represents those of BTX-treated muscle. Note that the latter models the principles of muscle tissue adaptations shown to occur experimentally for the rat EDL muscle (Ates and Yucesoy, 2014). An assumption was made that the adapted ECM material properties remain representative of the long-term-BTX and post-BTX treatment cases in an identical way. The stresses are normalized and dimensionless. The reader is referred to a review on LFMM model for full details (Yucesoy and Huijing, 2012).