Development of an Agent-Based Model to Study the Mechanism of Effects of Botulinum Toxin on Muscle Tissue Adaptation

Botulinum Toksinin Kas Dokusu Adaptasyonu Etkisinin Mekanizmasının Anlaşılması İçin Ajan-Tabanlı Model Geliştirilmesi

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***Abstract*— Local application of botulinum toxin type-A (BTX-A) is commonly used for spasticity management in patients with cerebral palsy (CP), stroke, multiple sclerosis, as well as dystonia. However, recent studies on muscle mechanics have shown results contradictory to treatment aims (increased passive resistance, narrower joint range of motion). This is ascribed to muscle structure adaptations characterized by collagen increase, hereby this should be controlled. However, the mechanisms of these adaptations are not understood. Within this study, it is aimed to develop an agent-based model to understand these mechanisms. Based on our previous finite element analysis results, the effects of BTX-A were modeled as strain changes across the extracellular matrix of the muscle fascicle. The analyzes performed by agent-based models showed statistically significant increases in the collagen turnover, the collagen sum and the number of fibroblasts due to BTX-A.**

Keywords — Cerebral palsy; Botulinum toxin type-A; Agent-based modelling; Muscle structure; Fibroblasts.

***Özetçe* — Botulinum toksin tip-A'nın (BTX-A) lokal uygulaması, serebral palsi (SP), felç, multiple-skleroz ve distoni hastalarında, spastisite yönetimi için yaygın olarak kullanılmaktadır. Ancak, kas mekaniği üzerine yapılan son çalışmalar, BTX-A'nın tedavi amaçlarıyla çelişen etkileri olduğunu göstermiştir (pasif direncin artması, eklem hareket aralığının daralması). Bulgular, kolajen artışı ile karakterize kas yapısı adaptasyonlarıyla açıklanmaktadır, dolayısıyla kontrol altına alınmalıdır. Ancak, bu adaptasyonların mekanizması anlaşılmamıştır. Bu çalışmada, ilgili mekanizmaları anlamak için ajan-tabanlı bir model geliştirilmesi amaçlanmıştır. Bu modelde önceki sonlu elemanlar analiz sonuçlarımıza dayanarak, BTX-A'nın etkileri, kas fasikülünün hücre dışı matrisi boyunca gerinim değişimleri olarak temsil edilmiştir. Ajan-tabanlı modeller ile yapılan analizler, kolajen dönüşümü, toplam kolajen ve fibroblast sayısında BTX-A sebebiyle istatistiksel anlamlı artışlar olduğunu göstermiştir.**

Anahtar Kelimeler — Serebral palsi; Botulinum toksin tip-A; Ajan-tabanlı modelleme; Kas yapısı; Fibroblast.

I. INTRODUCTION

Local application of botulinum toxin type-A (BTX-A) is commonly used for spasticity management in patients with cerebral palsy (CP), stroke, multiple sclerosis, as well as dystonia. BTX-A impairs the release of acetylcholine at the neuromuscular junction and reduces muscle hyperactivity and tonus by chemodenervation.
Treatment aims include a reduced passive resistance of the muscle at the joint and an improved joint range of motion. However, contradictory to those, recent studies showed that exposure to BTX-A causes an increased passive force, an increased muscle stiffness and a decreased length range of force exertion [1-2]. Such unintended BTX-A effects are ascribable to an observed increase in collagen content in the extracellular matrix (ECM) of the muscle exposed [2]. Therefore, these findings indicate that exposure to BTX-A leads to muscle tissue adaptation and altered mechanical properties of the ECM.

For an understanding of how ECM adaptations occur and eventually understand their effects on the structural and mechanical properties of the muscle, one approach is to understand interactions between muscle components due to BTX-A such as muscle fibers, fibroblasts and chemokines on a micro level. In order to achieve that, we aimed at developing an agent-based model of muscle exposed to BTX-A and to utilize its ability to simulate interactions in the cell level allowing to display the effect of their combined emergent behavior on the system as a whole. This approach has been successfully implemented in a muscle atrophy model by Martin et al. [3]

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and in an ECM-cell interaction model by Reinhardt et al. [4] to simulate biological systems and display mechanical interactions within biological structures.

II. METHODS

In order to understand the mechanisms of adaptation of the ECM, the Netlogo software [5] was utilized. As a base model, the muscle atrophy model developed by Martin et al. [3] was utilized. The atrophy model provides an excellent example of how simple rules and interactions between the muscle components and environment can lead to major changes on the behavior of cells, chemokine levels and ECM structure. That model focuses on muscle atrophy due to disuse but provides all the basic elements of a muscle fascicle cross-section from the contractile elements and ECM to the fibroblasts and chemokines necessary to study the effects of BTX-A on the inner workings of the skeletal muscle. Based on this approach, we developed a cross-sectional model of a fascicle (Fig. 1).

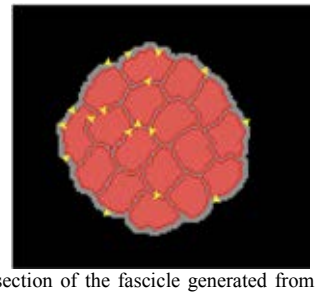

Figure 1. A cross section of the fascicle generated from the muscle atrophy model by Martin et al. [3] and used later in the BTX-A simulations. The figure shows the muscle fibers (red), the fibroblasts (yellow triangles) and the ECM (grey patches surrounding the muscle fibers and fascicle)

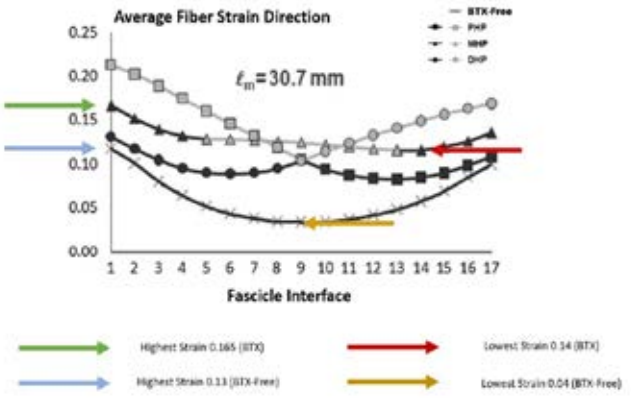

Figure 2. Average fiber strain of the BTX-A and BTX-Free cases as retrieved from the finite element modeling study of Türkoğlu et al. [6]. The finite element muscle model is comprised of 17 fascicles and the figure shows mean strain value per each of them for four different cases. For BTX-Free case, all fascicles are activated. For PHP, MHP and DHP cases, proximal, mid and distal halves, respectively of the muscle were not-activated to represent BTX-A induced partial muscle paralysis. The key message from this figure is that all BTX-A case curves are above that of BTX-Free case indicating a characteristic longer sarcomere effect. This is used presently to impose BTX-A effect into the developed agent based model.

In the present agent-based model, in order to impose the effects of BTX-A, the findings of an earlier finite element modeling study from our group was considered [6]. That study showed strain changes to occur within the muscle belly (Fig. 2), due to BTX-A: in short, BTX-A was shown to lead to a characteristic longer sarcomere effect because the paralyzed muscle parts did not shorten due to lack of muscle activation, and this effect was reflected also on the non-paralyzed muscle parts by the ECM limiting shortening due to activation. We used this strain effect as an input in our agent based model to impose the effect of BTX-A. Two cases were studied in both of which two fascicles modeled were compared:

Case 1 BTX-Free muscle-1 is represented by a fascicle that features strain values ranging between the maximum and minimum strain values from the BTX-Free strains as shown in Fig. 2 retrieved from Türkoğlu et al. [6]. *BTX muscle-1* is represented by a fascicle that features strain values ranging between the maximum and minimum strain values for the MHP BTX strains.

Case 2 BTX-Free muscle-2 is represented by a fascicle that involves zero strain applied to the ECM. *BTX muscle-2* is represented by a fascicle that features constant strain equal the difference between the difference between the maximums of the BTX-Free and BTX-A cases as shown in Fig. 2 (i.e., approximately 0.03 strain is imposed throughout the ECM).

For both cases the collagen turnover, which is the sum of collagen produced each time step (one hour) by all the fibroblasts in the muscle cross section, the collagen sum, which is the sum of collagen in the ECM, and the number of fibroblasts throughout the simulation time were all measured as parameters to compare the physiology of the muscle fascicle with and without the effects of BTX-A.

In order to ensure consistency of data, each fascicle in each case was simulated for 30 runs and each run was simulated for 720 hours (one-month) which is a duration suitable for the long term studies currently conducted in our project. One-way ANOVA was used to check the significance of the results.

III. RESULTS

Case 1 Fig. 3 shows the means of the collagen turnover, collagen sum and fibroblast count for 30 simulations for each fascicle representing the *BTX-Free muscle-1* and *BTX muscle-1* models. A noticeable and significant increase in all measured parameters can be seen in the muscle exposed to BTX-A.

Case 2 Fig. 4 shows the means of the collagen turnover, collagen sum and fibroblast count for 30 simulations for each fascicle representing the *BTX-Free muscle-2* and *BTX muscle-2* models. Again, a noticeable and significant increase in all measured parameters can be seen however with a bigger difference between the cases than the first case.

IV. DISCUSSION

It has become almost an established fact that fibroblasts within the muscle tissue respond, react and alter their behavior based on mechanical stimulations and changes in their environment

[7-9]. In addition to that, the paralytic effects of BTX-A have been found to cause mechanical changes within the muscle represented by strain changes. Hence, a clear line can be drawn between the paralytic effects of BTX-A and fibroblast activity. Accordingly, it is tenable to model the effects of BTX-A as strain changes across the ECM of the muscle fascicle.

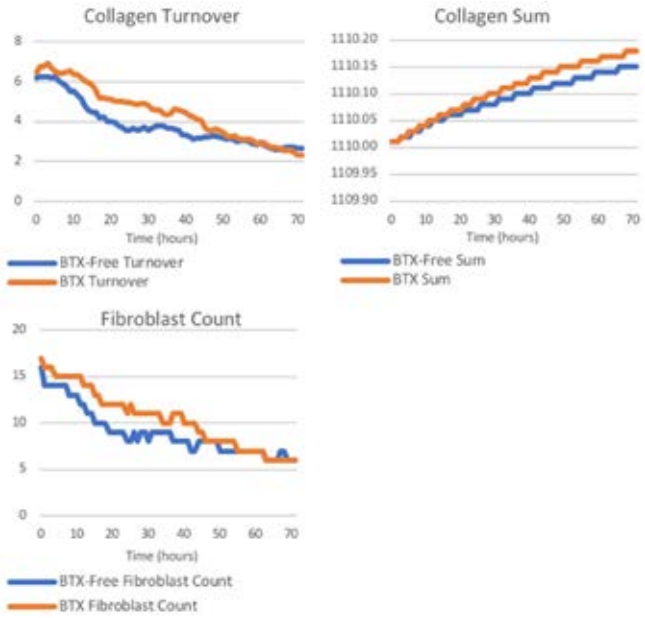

Figure 3. The collagen turnover (P=0.0027), collagen sum (P=0.026), and fibroblast count (P=0.0026), for the BTX-A and BTX-Free fascicles for Case 1.

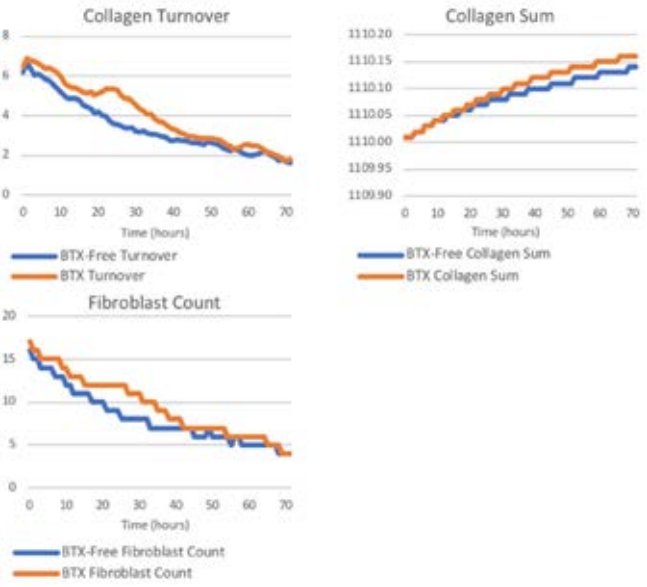

Figure 4. The collagen turnover (P=0.0015), collagen sum (P=0.049), and fibroblast count (P=0.0014), for the BTX-A and BTX-Free fascicles for Case 2.

Strain affects not only the fibroblasts themselves, but also the environment around them such as the chemokine secretions, which contribute hugely to the proliferative and apoptotic behavior of fibroblasts. Consequently, there was a significant increase in collagen turnover in the BTX-A muscles for most of the simulation time. This is attributed to increased activity and proliferation of fibroblasts as a result of strain which as shown in a number of studies [10-11]. Eventually, this led to an increase in the collagen sum throughout the ECM.

To conclude, an agent-based model of muscle exposed to BTX-A has been developed to the best of our knowledge for the first time in the literature. The first implementation of this model did show an increased collagen content, which is explained by the increase in activity of fibroblasts due to the BTX-A induced strain, which is imposed.

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REFERENCES

[1] Ateş, F., & Yucesoy, C. (2018). Botulinum toxin type-A affects mechanics of non-injected antagonistic rat muscles. Journal Of The Mechanical Behavior Of Biomedical Materials, 84, 208-216. doi: 10.1016/j.jmbbm.2018.05.027

[2] Ateş, F., & Yucesoy, C. (2014). Effects of botulinum toxin type A on non-injected bi-articular muscle include a narrower length range of force exertion and increased passive force. Muscle & Nerve, 49(6), 866-878. doi: 10.1002/mus.23993

[3] Martin, K., Blemker, S., & Peirce, S. (2015). Agent-based computational model investigates muscle-specific responses to disuse-induced atrophy. Journal Of Applied Physiology, 118(10), 1299-1309. doi: 10.1152/japplphysiol.01150.2014

[4] Reinhardt, J., Krakauer, D., & Gooch, K. (2013). Complex Matrix Remodeling and Durotaxis Can Emerge From Simple Rules for Cell-Matrix Interaction in Agent-Based Models. Journal Of Biomechanical Engineering, 135(7), 071003. doi: 10.1115/1.4024463

[5] Wilensky, U. (1999). NetLogo. http://ccl.northwestern.edu/netlogo/.

[6] Türkoğlu, A., Huijing, P., & Yucesoy, C. (2014). Mechanical principles of effects of botulinum toxin on muscle length–force characteristics: An assessment by finite element modeling.

[7] Hara, M., Fujii, T., Hashizume, R., & Nomura, Y. (2013). Effect of strain on human dermal fibroblasts in a three-dimensional collagen sponge. Cytotechnology, 66(5), 723–728. doi:10.1007/s10616-013-9638-3

[8] Yu, H., Kim, J., Kim, H., Lewis, M., & Wall, I. (2016). Impact of mechanical stretch on the cell behaviors of bone and surrounding tissues. Journal of Tissue Engineering, 7, 204173141561834. doi: 10.1177/2041731415618342

[9] Eastwood, M., McGrouther, D., & Brown, R. (1998). Fibroblast responses to mechanical forces. Proceedings Of The Institution Of Mechanical Engineers, Part H: Journal Of Engineering In Medicine, 212(2), 85-92. doi: 10.1243/0954411981533854

[10] Sun, L., Qu, L., Zhu, R., Li, H., Xue, Y., Liu, X., … Fan, H. (2016). Effects of Mechanical Stretch on Cell Proliferation and Matrix Formation of Mesenchymal Stem Cell and Anterior Cruciate Ligament Fibroblast. Stem cells international, 2016, 9842075. doi:10.1155/2016/9842075

[11] Koike, M., Shimokawa, H., Kanno, Z., Ohya, K., & Soma, K. (2005). Effects of mechanical strain on proliferation and differentiation of bone marrow stromal cell line ST2. Journal Of Bone And Mineral Metabolism, 23(3), 219-225. doi: 10.1007/s00774-004-0587-y