Clinical and Experimental Health Sciences

Original Article / *Özgün Araştırma*

The Effects of Intra-Articular Injections of Botulinum Toxin A on Experimentally Temporomandibular Joint Osteoarthritis Models

Botulinum Toksin A'nın İntraartiküler Enjeksiyonlarının Deneysel Temporomandibular Eklem Osteoartrit Modelleri Üzerine Etkileri

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Abstract

Objective: The aim of this study is to investigate the changes of temporomandibular joint (TMJ) by experimentally induced osteoarthritis (OA) and the effectiveness of botulinum toxin A (BTX-A) treatment via histopathological and biochemical methods.

Methods: Sprague-Dawley rats, aged 8 to 10 weeks, were used in this study. Intra-articular monosodium iodoacetate (MIA) solution was injected bilaterally into two experimental groups to induce TMJ osteoarthritis. Sterile 0.9% NaCl solution was injected to TMJ of healthy control group bilaterally to investigate the effect of trauma by intra-articular injection. Development of experimental osteoarthritis took 4 weeks, and blood samples were harvested from MIA group and examined to prove the presence of induced osteoarthritis. OA-induced eight rats with BTX-A. Another OA-induced eight rats were classified as a control group and intra-articular 0.9% NaCl solution was injected bilaterally. All samples were sacrificed 6 weeks after treatment. Blood samples and specimen of TMJ were harvested from all rats and examined histopathologically and biochemically.

Results: BTX-A and 0.9% NaCl solution groups showed some improvement compared with untreated MIA group but it was not statistically significant. There was no sign of trauma in 0.9% NaCl solution-injected healthy group. Pathological findings were also consistent with the biochemical results.

Conclusion: BTX-A was not successful at treatment of OA and is not a proper selection especially when used intra-articularly.

Keywords: Botulinum toxin, monosodium iodoacetate, osteoarthritis, tempora mandibuler joint

Öz

Amaç: Bu çalışmanın amacı, deneysel olarak indüklenen osteoartrit (OA) ile temporomandibular eklemin (TME) değişikliklerini ve histopatolojik ve biyokimyasal yöntemlerle botulinum toksin A'nın (BTX-A) etkinliğini araştırmaktır.

Yöntemler: Bu çalışmada 8 ila 10 haftalık Sprague dawley fareleri kullanılmıştır. TME osteoartritini indüklemek için iki deney grubuna iki taraflı olarak intraartiküler monosodyum iyodoasetat (MIA) solüsyonu enjekte edilmiştir. İntraartiküler enjeksiyon ile travmanın etkisini araştırmak için sağlıklı kontrol grubunun TME'sine steril %0,9'luk NaCl solüsyonu ikili olarak enjekte edilmiştir. Deneysel osteoartritin gelişimi 4 hafta sürdü ve kan örnekleri MIA grubundan elde edildi ve indüklenen osteoartrit varlığını ispatlamak için incelendi. OA, BTX A ile 8 sıçanı indüklemiştir. OA ile indüklenen bir başka sıçan kontrol grubu olarak sınıflandırılmış ve bilateral eklem içi %0,9 NaCl solüsyonu enjekte edilmiştir. Tüm numuneler, tedaviden 6 hafta sonra sakrifiye edilmiştir. Kan örnekleri ve TMJ örneği tüm sıçanlardan hasat edildi ve histopatolojik ve biyokimyasal olarak incelenmiştir.

Bulgular: Gruplar. BTX-A ve %0,9 NaCl çözelti grupları, tedavi edilmemiş MIA grubuna kıyasla bir miktar iyileşme gösterdi, ancak istatistiksel olarak anlamlı değildir. %0.9 NaCl solüsyonu enjekte edilen sağlıklı grupta travma belirtisi yoktur. Patolojik bulgular aynı zamanda biyokimyasal sonuçlar ile de uyumluydu.

Sonuç: BTX-A, OA tedavisinde başarılı değildi ve özellikle de intraartiküler kullanıldığında uygun bir seçim değildir.

Anahtar Kelimeler: Temporomandibular eklem, osteoartrit, botulinum toksini, monosodyum iyodoasetat

INTRODUCTION

Stomatognathic system consists of muscles of head and neck, masticatory muscles, ligaments, temporomandibular joint (TMJ), teeth, cheek, lips, and salivary glands (1-3). The long-term health of the TMJ, which is a synovial type of a joint, is influenced by efficacy of the mechanisms that control stress on the articular surfaces. Synovial membrane, synovial fluid, disc, and fibrocartilage (joint cartilage) play a role in joint lubrication (4). Many factors are responsible for TMJ dysfunction. One of the most commonly accused etiological factor is occlusion disorder (1, 4, 5).

Botulinum toxin A (BTX-A) application to masseter or temporal muscle as a treatment option in TMJ disorders has been reported to reduce pain and dysfunction and help increase mouth-opening range (6, 7).

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Because of its close association with bone destruction, inflammation, and degenerative tissue changes, we have preferred to use TNF-α (Tumor necrosis factor-alpha) levels to demonstrate development of OA in our study (8).

Available experimental OA models include surgical models, mechanical OA model, drug-induced OA model, and spontaneous OA (9-14). The monosodium iodoacetate (MIA) model of OA that we used in the present study has been used many times in the literature and has a proven effect (15).

METHODS

The study was conducted on 28 male Sprague-Dawley strain rats, aged between 8 and 10 weeks, weighing 228 grams on average, which were reproduced in İstanbul University Experimental Medicine Research Institute Laboratory. The experiments and postoperative care were conducted in the same laboratory. The study was approved by Istanbul University Animal Experiments Local Ethics Committee. Pathological examination of specimens obtained from the animals were performed in İstanbul University Faculty of Medicine, Pathology Department, and biochemical analyses of blood samples were performed in İstanbul University Cerrahpaşa Faculty of Medicine, Biochemistry Department.

In order to determine serum TNF-α levels in rats that do not have induced OA, a reference group (Group I) was created consisting of eight rats, and intra cardiac blood samples were obtained at the beginning of the experiment. The remaining groups of animals were put to cages with four animals in each cage. They were fed and water was provided ad libitum with standard laboratory rat feed in standard laboratory environment at 22°C±1°C room temperature, with 12 hours of light and dark cycle.

In order to induce experimental OA, 1 mg/kg monosodium iodoacetate (MIA, Sigma, Saint Louis, USA) diluted in 50 µL 0.9% NaCl solution was injected to upper compartment of bilateral TMJ of rats in Groups I, II, and III, using 27-gauge 0.5-inch injector, as described by Wang et al. (15) in 2012. In addition to these groups, another group was formed composed of seven rats (Group IV, sham treatment), and only intra-articular 0.9% NaCl solution was injected to the rats in this group without inducing OA. We waited 4 weeks for the development of experimental osteoarthritis. At the end of the fourth week, intra cardiac blood samples obtained from rats in Group I were sent for biochemical analysis. To demonstrate OA development, TNF-α levels in these blood samples were analyzed with RayBio® Rat TNF-α ELISA (Enzyme-Linked Immunosorbent Assays) Kit, according to manufacturer's instructions. In Groups I, II, and III, which had experimentally induced bilateral TMJ OA, two different substances were administered to animals via intra-articular injections for treating TMJ OA as follows: 5 units/kg BTX-A diluted in 50 µL 0.9% NaCl solution in Group II. After a predetermined treatment period of 6 weeks, all animals were sacrificed, via drawing intra cardiac blood.

Blood samples were centrifuged, and serum was separated and stored at -70°C temperature until time of biochemical analysis. TMJ was dissected in all rats, paying attention not to give any harm to the joint disc and condyle. Dissected joints were fixated in 10% formaldehyde solution and sent for pathological examination. For the measurement of TNF-α levels in experiment (Groups II and III) and control (Groups I and IV) groups, the serum samples were brought to room temperature and analyzed using RayBio® Rat TNF-α ELISA Kit and standard ELISA plates according to manufacturer›s instructions.

TMJ specimens were kept in 10% formaldehyde solution for 2 weeks and then in Bouin's solution for 2 days. After this fixation procedure, the specimens were decalcified in a solution containing 10% acetic acid, 0.85% NaCl, and 10% formalin. They were then embedded in paraffin blocks and cut in longitudinal sections of 3-4 µm thickness. Sections were stained with hematoxylin and eosin and examined under a light microscope.

RESULTS

Biochemical Results

The mean serum TNF-α level of the healthy rats in the reference group that did not have induced OA was 6898.8571 pg/ml, and the mean serum TNF-α level of the rats that were sacrificed after OA development at the end of 4 weeks, without receiving any intra-articular injection to their TMJ for treatment (Group I), was 16828,0000 pg/mL. At the end of the 10th week of the experiment, mean serum TNF-α levels were 10976,0000 pg/mL in BTX-A group (Group II) and 10813,0000 pg/mL in the sterile injection group (Group III).

Mean serum TNF-α level of the group that received only 0.9% NaCl solution injection without induction of OA to determine the effects of the trauma caused by intra-articular injection and were kept for 6 weeks as the rest of the treatment groups (Group IV) was 7418.8571 pg/ml (Table 1).

Statistical analyses of biochemical and pathological findings in all groups were performed with ANOVA (Analysis of Variance) test, and since there were more than two groups, comparisons between groups were made with Bonferroni test**.**

Table 1. Mean serum TNF-α levels in study groups

Group	TNF-α (pg/mL)
Healthy reference	6898.8571
MIA	16828,0000
MIA+BTX-A	10976,0000
MIA+0·9% NaCl soluti on	10813,0000
Healthy animal (0.9% NaCl solution)	7418.8571
MIA: Monosodyum iyodoasetat; BTX A: Botulinum toksin a; Nacl: Sodium chloride; TNF-a: Tumor necrosis factor alpha	

Table 2. Pathological findings in experimental group

Group	Number of TMJ's with cartilage lesion
MIA	12
MIA+BTX-A	6
MIA+0.9% NaCl solution	2
Healthy animal (0.9% NaCl solution)	0
MIA: Monosodyum iyodoasetat; BTX-A: Botulinum toksin A; TMJ: Temporomandibular joint; NaCl: Sodium chloride	

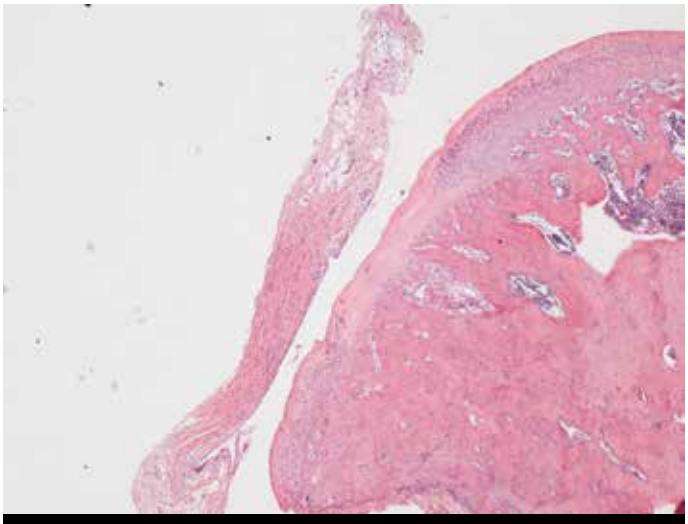

Figure 1. Area of cell loss in cartilage tissue at the condylar surface, and its magnified view HE 40×

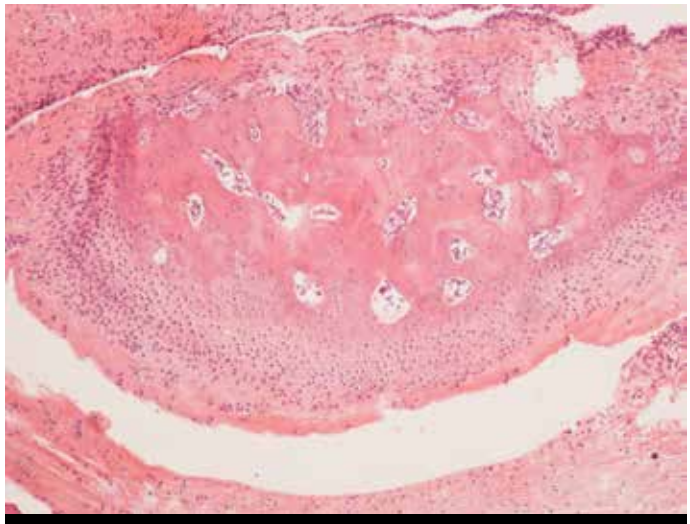

Figure 3. Area of cell loss in cartilage tissue at the condylar surface HE 200×

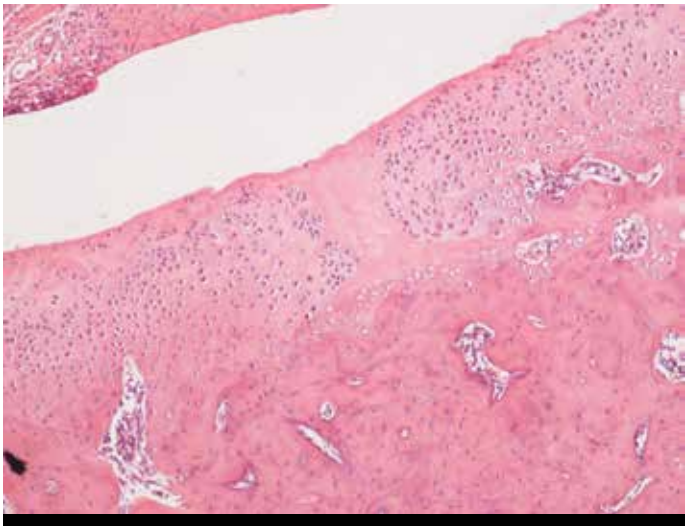

Figure 2. Area of cell loss in cartilage tissue at the condylar surface HE 100×

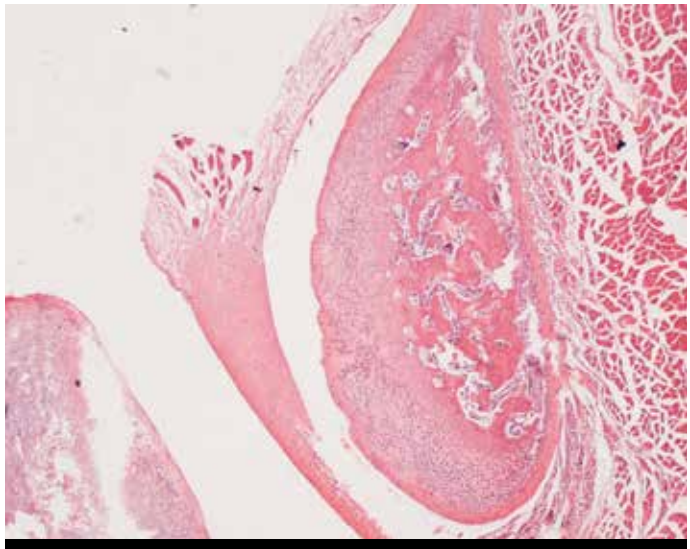

Figure 4. Joint with no damage at its condylar surface HE 40×

According to the results, mean serum TNF-α level in MIA group (16828,0000 pg/mL) was significantly higher compared to healthy reference group (6898.8571pg/mL) and the group of healthy animals that only received 0.9% NaCl solution injection (7418.8571 pg/mL) ($p<0.05$).

Mean serum TNF-α level in the group of healthy animals that only received 0.9% NaCl solution injection (7418.8571 pg/mL) was significantly lower than MIA group (16828,0000 pg/mL) ($p<0.05$). For comparison of mean serum TNF-α levels between MIA group and 0.9% NaCl solution group, $p = 0.015$. No significant difference was found in other groups.

Histopathological Findings

Four weeks after MIA injection, rats in Group I were sacrificed and the dissected TMJs were examined histopathologically. Accordingly, 12 of 16 joint cartilage tissues obtained from eight animals showed cartilage lesions (degeneration), which are areas of cell loss (Figure 1).

In Group II, after 4 weeks from MIA injection, the rats were treated with intra-articular BTX-A injection. At the end of 6 weeks' treatment period, rats were sacrificed at the end of 10th week and their TMJs were examined. Accordingly, 6 of 16 joint cartilage tissues obtained from eight animals showed cartilage lesion, that is, areas of cell loss (Figure 2).

In Group III, after 4 weeks from MIA injection, the rats were treated with intra-articular 0.9% NaCl solution injection. At the end of 6 weeks' treatment period, rats were sacrificed at the end of 10th week and their TMJs were examined. Accordingly, 2 of 16 joint cartilage tissues obtained from eight animals showed cartilage lesion, that is, areas of cell loss (Figure 3).

In Group IV that comprised seven healthy rats that did not receive MIA injection, intra-articular 0.9% NaCl solution injection was made at the end of 4th week, and the rats were sacrificed 6 weeks later at the end of 10th week and their TMJs were examined. Accordingly, none of the 14 joint cartilage tissues obtained from seven rats were found to have cartilage lesion (Figure 4) (Table 2).

The number of TMJs with cartilage lesion in the group that had induced OA with MIA injection (Group I) was significantly higher com-

pared to reference group (Group II), MIA + 0.9% NaCl solution group (Group III) and the group of healthy animals that only received 0.9% NaCl solution injection (Group VII) ($p<0.05$).

Correlation of histopathological cartilage lesion findings with serum TNF-α levels was evaluated. Accordingly, Pearson's correlation coefficient was +0.711, and the correlation was statistically significant ($p>0.000$).

DISCUSSION

Current treatment methods of TMJ disorders are not capable to eliminate patients' complaints completely. OA is a progressive disease that has various etiopathogenetic reasons. The main target in its treatment is to alleviate pain, ease joint motions, increase mouth-opening range, and eliminate functional disturbance. Different conservative methods are used in its treatment. The aim of treatment is to procrastinate surgery requirement and reduce cartilage degeneration that starts from the early stages of OA but does not manifest clinical symptoms.

Prevalence of TMJ OA increases with age. Intra-articular injections are most commonly preferred for treatment of TMJ OA (16). Various preparations can be used intra-articularly in TMJ OA and BTX-A that is injected to masseter and temporal muscles under EMG guidance (6, 7, 17, 18).

In our study, we induced experimental OA in TMJ of rats. After biochemical demonstration of OA development, we performed intra-articular injections with BTX-A, and we compared their histopathological and biochemical effects on the course of disease with each other and in reference to control groups. Experimental OA was induced by bilateral intra-articular MIA injection to TMJ (15). Then, blood samples were obtained from the diseased group that did not receive any treatment, and OA development was demonstrated biochemically by the elevated serum TNF-α levels in these rats. After 6 weeks of treatment period, all the remaining groups were sacrificed.

We found mean serum TNF-α level in the group treated with bilateral intra-articular HA injection after chemical OA induction with MIA as 7693.1250 pg/mL. Mean serum TNF-α level in the group that had induced OA but did not receive treatment was 16828.0000 pg/ml. The reduction in serum TNF-α level seen after treatment was statistically significant.

Histopathological findings were also consistent with this result. Mean serum TNF-α level in the group treated with BTX-A was 10976,0000 pg/mL. Mean serum TNF-α level in the group that had induced OA but did not receive treatment was 16828.0000 pg/ml. Although there was a reduction in TNF-α levels with treatment, the difference was not statistically significant. In histopathological examination, 6 of 16 TMJs were found to have cartilage lesion and area of cell loss. On the basis of these results, it was concluded that intra-articular BTX-A application was ineffective and that the reduction in serum TNF-α level was not due to therapeutic effect of BTX-A but might be related to spontaneous healing mechanism that occurred during the 10 weeks after disease onset.

In order to determine the effects of the trauma caused by the cannula used to administer intra-articular injections, we administered only 0.9% NaCl solution to a group of healthy rats that did not have induced OA. In this group, although mean serum TNF-α level was found to be slightly higher in comparison to healthy animals, the difference was not statistically significant. Similarly, pathological examination of their TMJ did not reveal any damage to the cartilage. Therefore, it was concluded that the injection procedure itself did not cause any significant mechanical trauma to the joint.

CONCLUSION

In the present study, we induced bilateral experimental OA in TMJ of male Sprague-Dawley strain rats and compared the efficacies of treatment with BTX-A.

The mean TNF-α level in BTX-A group was lower than in the diseased group. However, the difference was not statistically significant. According to histopathological findings, BTX-A group showed the lowest success. On the basis of these results, it can be concluded that BTX-A is unsuccessful in treating OA.

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REFERENCES

1. Badel T, Ćimić S, Munitić M, Zadravec D, Kes VB, Šimunković SK. Clinical view of the temporomandibular joint disorder. Acta Clin Croat 2014; 53: 462-70.
2. Heffez J, Mafee MF, Rosenberg HM. Atlas of Temporomandibular Joint Imaging. UK: Lippincott Williams and Wilkins; 1995.
3. Okeson JP. Management of Temporomandibular Disorders and Occlusion. 2nd ed. St. Louis: Mosby; 2007.
4. Singh RK. Temporomandibular joint disorders. Natl J Maxillofac Surg. 2016; 7: 1-2. [CrossRef]
5. Bourbon B. Craniomandibular Examination and Treatment. RS. Myers Saunders Manual of Physical Therapy Practice. Philadelphia: W.B. Saunders Co; 1995.

6. Freund B, Schwartz M. The use of botulinum toxin for the treatment of temporomandibular disorder. Oral Health. 1998; 88: 32-7.
7. Freund BJ, Schwartz M. Intramuscular injection of botulinum toxin as an adjunct to arthrocentesis of the temporomandibular joint: preliminary observations. Br J Oral Maxillofac Surg. 2003; 41: 351-2. [CrossRef]
8. Jorgensen NR. Cytokines and osteoporosis. Ugeskr Laeger. 1997; 160: 24-8.
9. Meng J, Ma X, Ma D, Xu C. Microarray analysis of differential gene expression in temporomandibular joint condylar cartilage after experimentally induced osteoarthritis. Osteoarthritis Cartilage 2005; 13: 1115-25. [CrossRef]
10. Fujisawa T, Kuboki T, Kasai T, Sonoyama W, Kojima S, Uehara J. et al. A repetitive, steady mouth opening induced an osteoarthritis-like lesion in the rabbit temporomandibular joint. J Dent Res. 2003; 82: 731-5. [CrossRef]
11. Xinmin Y, Jian H. Treatment of temporomandibular joint osteoarthritis with viscosupplementation and arthrocentesis on rabbit model. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005; 100: 35-8. [CrossRef]
12. Cledes G, Felizardo R, Foucart JM, Carpentier P. Validation of a chemical osteoarthritis model in rabbit temporomandibular joint: a compliment to biomechanical models. Int J Oral Maxillofac Surg 2006; 35: 1026-33. [CrossRef]
13. Wadhwa S, Embree MC, Kilts T, Young MF, Ameye LG. Accelerated osteoarthritis in the temporomandibular joint of biglycan/fibromodulin double-deficient mice. Osteoarthritis Cartilage. 2005; 13: 817-27. [CrossRef]
14. Ghassemi Nejad S, Kobezda T, Tar I, Szekanecz Z. Development of temporomandibular joint arthritis: The use of animal models. Joint Bone Spine 2017; 84: 145-51. [CrossRef]
15. Wang XD, Kou XX, He DQ, Zeng MM, Meng Z, Bi RY, et al. Progression of cartilage degradation, bone resorption and pain in rat temporomandibular joint osteoarthritis induced by injection of iodoacetate. PLoS One 2012; 7: 45036. [CrossRef]
16. Guarda-Nardini L, Manfredini D, Stifano M, Staffieri A, Marioni G. Intra-articular injection of hyaluronic acid for temporomandibular joint osteoarthritis in elderly patients. Stomatologija 2009; 11: 60-5.
17. Guarda-Nardini L, Masiero S, Marioni G. Conservative treatment of temporomandibular joint osteoarthrosis: intra-articular injection of sodium hyaluronate. J Oral Rehabil 2005; 32: 729-34. [CrossRef]
18. Kurtoglu C, Gur OH, Kurkcu M, Sertdemir Y, Guler Uysal F, Uysal H. Effect of botulinum toxin-A in myofascial pain patients with or without functional disc displacement J Oral Maxillofac Surg 2008; 66: 1644-51. [CrossRef]