The Benefits of Botulinum Neurotoxin Treatment in a Multitude of Medical Conditions

TIBERIU PAUL NEAGU[1,2], ION COCOLOS[3], CRISTIAN COBILINSCHI[4], MIRELA TIGLIS[4]*, IOAN PETRE FLORESCU[1,5], ELISABETA BADILA[6,7], RUXANDRA DIANA SINESCU[1,2]

[1]Carol Davila University of Medicine and Pharmacy Bucharest, Clinical Department No. 11, 8 Eroii Sanitari, 050474, Bucharest, Romania

[2]Emergency Clinical Hospital of Bucharest, Department of Plastic Surgery and Reconstructive Microsurgery, 8 Calea Floreasca, 014461, Bucharest, Romania

[3]Emergency Clinical Hospital of Bucharest, Department of Orthopedics, 8 Calea Floreasca, 014461, Bucharest, Romania

[4]Emergency Clinical Hospital of Bucharest, Department of Anesthesiology and Intensive Care, 8 Calea Floreasca, 014461, Bucharest, Romania

[5]Bagdasar Arseni Emergency Clinical Hospital of Bucharest, 12 Berceni Road, 041915, Bucharest, Romania

[6]Carol Davila University of Medicine and Pharmacy Bucharest, Clinical Department No. 5, 8 Eroii Sanitari, 050474, Bucharest, Romania

[7]Emergency Clinical Hospital of Bucharest,Department of Internal Medicine, 8 Calea Floreasca, 014461, Bucharest, Romania

Botulinum neurotoxins (BoNTs) are responsible for botulism in humans and vertebrates, being one of the six most catastrophic potential bioterrorism agents. This are ~150 kDa proteins, assembled as a ~50 kDa light chain (LC) and a ~100 kDa heavy chain (HC). The LC acts like a zinc metalloproteinase that cleaves three proteins in neurons, members of the SNARE (Soluble N-ethylmaleimide sensitive fusion attachment protein receptors) family: VAMP (vesicle-associated membrane protein) / synaptobrevin, SNAP-25 (synaptosomal-associated protein 25) and syntaxin. After cleavage of any of this proteins, neurotransmission is blocked and flaccid paralysis of the muscle is installed. This extraordinary restricted tropism for the cholinergic presynaptic membrane makes this drug unique regarding its toxicity, pharmacological and therapeutic use. Taking into consideration the potential of this substance, this paper aims to summarize the most relevant data regarding the mechanism of actions and its main clinical applications, in order to improve medical practice. Therefore, we presented the mechanism of action in order to understand its usage in different pathologies, such as dystonias, spasticity, nephrologic and urologic conditions, cosmetic use, depression, gastroenterologic and proctologic diseases, dermatologic conditions, pathologies specific to plastic surgery and also the role of BoNT therapy in pain management. It is well documented in the literature that important discoveries have been made through recent experimental and clinical studies. Even so, there is still much to learn about the therapeutic action of this drug in terms of molecular and pathophysiological mechanisms, in order to benefit of the whole healing potential of this amazing toxin.

Key words: botulinum toxin, movement disorders, pain management, neurotransmission blockage, zinc metalloproteinase.

Botulinum neurotoxins (BoNTs) are responsible for botulism in humans and vertebrates, being one of the six most catastrophic potential bioterrorism agents [1]. However, in small quantities and targeted administration, this toxins are responsible for blocking specific targets in order to treat different medical conditions, proving to be of great use in medicine [1]. There are seven major types (from A to G), produced by various strains of clostridia bacteria in the absence of oxygen, as a ~50 kDa light chain (LC) and a ~100 kDa heavy chain (HC) assembled in ~150 kDa proteins [1]. The HC contains two domains responsible for transportation (N-terminal) and for receptor-binding (C-terminal). The LC has a tetrahedral zinc binding that acts like a metalloproteinase which cleaves three proteins in neurons, members of the SNARE (Soluble N-ethylmaleimide sensitive fusion attachment protein receptors) family: VAMP (Vesicle–associated membrane protein) / synaptobrevin, SNAP-25 (Synaptosomal-associated protein 25) and syntaxin. After cleavage of any of this proteins, neurotransmission is blocked and flaccid paralysis of the muscle is installed. Affinity of binding and extraordinary restricted tropism for the presynaptic membrane of the cholinergic peripheral nerve, together with the catalytic proprieties, makes this substances unique regarding their toxicity, pharmacological and therapeutic use [1,2]. After first being used in ophthalmology with the aim to relieve hypercontraction of the eyelid small muscles, the neurologists used it to treat hemifacial spasm, dystonic muscles of the cervical region and later to treat limb movement pathologies and limb spasticity. Due to the fact that BoNTs blocks not only the neuromuscular junctions, but also autonomic innervation which controls tear [3], salivary glands [4], sweat [5], smooth muscles and sphincters, this substances may be used in dermatology [6], nephrology [7-9], urology [10,11], rheumatology [12], gastroenterology [13,14], endocrinology [15-18], and others. Taking into consideration the extraordinary potential of this substance, this paper aims to summarize the most relevant data regarding the mechanism of actions and its main clinical applications, in order to improve medical practice for those who are interested in managing various diseases that may benefit from BoNT therapy.

* *e-mail: mirelatiglis@gmail.com; Phone +4021.599.23.00*

Mechanism of action

There are several steps that need to take place in order for the botulinum toxin to be activated. First, the carboxy-terminal end of the HC binds to a presynaptic polysialic ganglioside receptor of the membrane, respectively, a vesicular glycoproteins (SV2) for type A, E and synaptotagmin I and II (Syt) for type B, G and DC. Other cell-surface proteins may attach BoNT type A, such as E-cadherin, vanilloid receptors and Fibroblast Growth Factor Receptor 3 (FGFR3) [19]. Then, the toxin is internalized in different ways of SV endocytosis, depending on the receptors, using the proton pomp, inside synaptic vesicles [20]. In order for the effect of BoNT therapy to occur, the LC must be released into the cytosol, which follows membrane translocation of the LC disulfide linked to the HC. In order to release its zinc metalloprotease activity, the reduction of the disulfide(S-S) link between the light and the heavy chain of the toxin must take place at the cytosolic aspect of the membrane [21]. Reduction of the S-S bond prior to cytosol results in abortion of the L-chain translocation, therefore a low pH and an oxidizing lumen is required in order for this not to happen [21]. After activation, the LC zinc metalloprotease cleaves SNAP 25 (BoNT/A, BoNT/E), VAMP/synaptobrevin (BoNT/B, D, F, G) or both SNAP 25 and syntaxin (BoNT/C), thus, causing inhibition of acetylcholine release [19]. Cleavage of 10% to 15% of total SNAP-25 (spinal cord and neuromuscular junctions) is enough to determine paralysis [22]. Based on extended and various protein-protein binds with specific substrate, including cleavage site and exosites, the LC have great specificity for SNARE proteins. This leads to precise recognition of the substrate of the enzyme and explains why other proteins are not yet discovered to be targeted by the BoNT therapy [21].

BoNT receptors have been identified both in neuronal and non-neuronal cells [23]. Some non-neuronal cells expressing BoNT type A binding receptors (SV2, FGFR3, vanilloid receptors) are as follows: adipose mesenchymal cells, intestinal/prostate/urothelial/alveolar epithelial cells, epidermal keratinocytes, macrophages, neutrophils, T47D/MDA-MB-453/MDA-MB-231 breast cells, dermal fibroblast, sebocytes, mast cells and endothelial cells [23]. Therefore, the BoNT therapy may prove useful in different clinical conditions due to its variability of targeted cells lines.

Clinical applications

Dystonias

Local injections of BoNT mainly decreases the release of acetylcholine from alpha motoneurons, but also influences the activity of the gamma motoneurons, therefore, it is considered the first option for the treatment of focal, segmental dystonias and hemifacial spasm. HALLETT, M., et al. published a meta-analysis study regarding the success of BoNT therapy in movement disorders such as blepharospasm, cervical, focal limb, laryngeal and oromandibular dystonia, also hemifacial spasm [24].

In order to treat forced eyelid closure (blepharospasm), BoNT therapy may be applied on both sides to *orbicularis oculi*, *procerus* and *corrugator supercilii*. Several studies confirmed the efficacy of this treatment with a response rate of 90%, making this one of the most successful indications for BoNT therapy [24].

Also known as *spasmodic torticollis*, cervical dystonia may benefit from up to 300 U of Botox, 800 U of Dysport or 20000 U of Myobloc in order to reduce pain and impairment of head control (table 1) [24]. In case of focal limb dystonia (arm or leg), higher dosage may be required. In the upper extremity, musician`s and writer`s cramps are most frequently encountered, while in the lower limb, genetic dystonia or associated with Parkinson disease may be idiopathic or symptomatic. Usually, electromyographic or ultrasound guidance may improve accurate muscle selection and identification for BoNTs injection [25].

Tics, tremors or hemifacial spasm described as unilateral contractions, may benefit from BoNT therapy. Temporomandibular joint disorders or Meige syndrome (oromandibular dystonia and blepharospasm) are also associated with myalgia for which BoNT injection may improve the prognostic of the disease. However, in some cases, Botox therapy (Commercial name of the onabotulinumtoxin A – BoNT type A1) after arthroplasty for the residual myalgia was associated with mandibular condyle resorption [26]. It is believed that muscle contraction and exercised forces along bones, among other factors like the integrity of periosteum, osteosynthesis methods in fractured bones influence the balance between bone resorption and production [27-29]. Studies revealed that usually smaller doses than the ones required in blepharospasm are injected [24].

Spasticity

Spasticity is an association of central paresis and muscle abnormal activity such as dystonia, rigidity and spasm, followed often by pain [30]. It is developed usually, after cerebral strokes, spinal cord lesions, traumatic brain injury, infantile cerebral palsy or multiple sclerosis. The BoNT therapy aims to reduce muscle tone, prevent contractures and decubitus, facilitate nursing, improve function and relieve pain (table 1). The efficiency of this therapy is improved when associated with early rehabilitation programs [31].

Renal and urologic conditions

In nephrology, according to recent studies, the BoNT therapy may be used to treat drug resistant hypertension [32], to improve the blood flow of the dialysis fistulas[32,33], to relieve pain in peritoneal dialysis[34,35] or to reduce the amount of unnecessary pain medication for the patients following dialysis or with renal transplant[34,36,37]. LEE, S.H., et al. published a case where celiac plexus block was performed using 100 U of Botox in order to obtain a response to antihypertensive medication and to control blood pressure (table 1) [32]. Other studies, revealed that patients with diabetic peripheral neuropathy and nephropathy may benefit from BoNT therapy in order to reduce the associated pain without taking high doses of painkillers [37,38]. Further studies regarding the effects of ultrasonography guided BoNT therapy in order to reduce pain in lithiasic renal colic, on patients that could not tolerate conventional treatment may be of great use [39].

In urology, the BoNT therapy has proven to be beneficial for the treatment of detrusor sphincter dyssynergia, detrusor overactivity and symptoms of the lower urinary tract associated with benign hyperplasia of the prostate. These disorders are commonly encountered among patients with spinal cord pathologic conditions (traumatic injuries, multiple sclerosis, tumors). A recent study reported that 200 U of BoNT type A proved to be useful in treating neurogenic detrusor overactivity, while the short-term efficiency in treating idiopathic detrusor overactivity remains to be investigated (table 1) [10]. Smooth muscle relaxation of the prostate after using BoNT therapy is reliable in treating low tract urinary symptoms [11]. Besides its

effects on acetylcholine release, it is believed, according to basic and clinical evidence, that BoNT inhibits the release of substance P, calcitonin related peptide, glutamate, neurotrophins, ATP and cyclooxygenase-2 products from bladder neurons peripheral terminals and urothelium, improving pain control and function [40].

Among other urologic pathologies, according to a recent study, the BoNT therapy may improve responsiveness to medication in patients that suffer from vasculogenic erectile dysfunction [11]. In addition to other treatments that could improve sexual function [41], the cavernosal smooth relaxation due to the inhibitions of acetylcholine release allows effective compression of the emissary and subtunical veins in order to obtain a full penile erection [11]. In order to achieve responsiveness to phosphodiesterase type 5 inhibitors in non-responder patients, an intracavernosal administration of 50 U of BoNT type A was performed. Based on this evidence, in a phase II clinical study, conducted by GHANEM, H., et al., a higher dose of 100 U will be administered in order to see if it leads to a better response [11].

Gastroenterologic and Proctologic Conditions

Compared to other therapies, BoNT therapy has led to very good outcomes, in order to treat chronic anal fissures and spastic gastrointestinal disorders, especially achalasia. Endoscopic injection of Botox in all four lower esophageal sphincter quadrant is the most common choice in order to treat achalasia in the United States [42]. However, BoNT therapy has not been chosen as first-line therapy, compared to invasive dilation or Heller's myotomy which are associated with long lasting results [42]. On the other hand, in chronic anal fissures, BoNT therapy has become standard treatment, being involved not only in reducing symptomatology, but also in enhancing the healing process by reducing the sphincter pressure and influencing the nitric oxide synthase [14,43].

A recent review aimed to establish the role of BoNT therapy in obesity management, starting from the fact that ultrasound-guided endoscopic injections into the gastric wall may prolong gastric emptying and cause early satiety [13]. However, the published studies are lacking consistency regarding different aspects. Some studies, revealed that delaying gastric dumping and increasing satiety was not associated with weight loss [13]. Other studies revealed that this therapy provided loss of total body weight but it was associated with a restricted diet [13]. Therefore, this topic remains to be further investigated, even though preliminary studies in rodents proved that BoNT therapy is a valid approach in treating obesity [44].

Cosmetic Use

Facial muscle relaxation due to BoNT type A targeted delivery improves cutaneous elasticity, viscoelastic proprieties, pliability, organization and orientation of collagen fibers [23]. A recent meta-analysis underlined the fact that 20 U of Botox are extremely effective in treating glabellar lines [45]. In Europe, some products have received approval to be used in order to treat frontal frown lines and lateral periorbital lines. However, off-label BoNT therapy may be used to reduce all wrinkles of the skin associated with muscle tone, especially in the head and neck region. Best aesthetic results are achieved when BoNT therapy is associated with hyaluronic acid injections, mesotherapy or platelet-rich-plasma injections [46]. Even if it may be used in order to improve appearance, this therapy proved useful in treating depression after administration of 20 U of BoNT/A in the glabellar area [47].

Dermatologic Conditions

Besides the cosmetic application of BoNT therapy in dermatology, there are several conditions in which this treatment may prove useful. Type A toxin is used in skin diseases associated or worsened by hyperhidrosis such as chromhidrosis, hidradenitis suppurativa, Frey Syndrome, pompholyx, dyshidrosis, Hailey-Hailey disease, inverse psoriasis, Darier disease, pachyonychia congenital and aquagenic palmoplantar keratoderma [23]. This therapy improved life quality and pain management in leg ulcers, painful cutaneous leiomyomas and other skin tumors, lichen simplex, notalgia paresthetica, anal fissures and postherpetic neuralgia [5,23,48-52]. Due to type A BoNT effects on eccrine gland abnormalities, it has been used to improve outcomes in treating eccrine sweat gland nevi, congenital eccrine nevus, multiple eccrine hydrocystomas and eccrine angiomatous hamartoma [23]. Therapeutic activity was also revealed in treating androgenetic alopecia, IgA bullous dermatosis, alopecia areata, facial erythema, flushing and multiple forms of itching [6,23]. There are several clinical trials concerning BoNT therapy in different conditions, such as post-excisional scaring, scleroderma-associated Raynaud syndrome, localized vitiligo, herpes labialis, acne and oily skin [6,23].

The utility of BoNT therapy in Plastic Surgery

Among aesthetic considerations of this therapy, blocking different muscles and diminishing skin tension of different regions may improve scaring formation and prevent keloids/hypertrophic scars after various reconstructive or posttraumatic surgical procedures [6,23]. Different studies proved that itching associated with burn lesions may be treated using BoNT therapy [6,23,53]. Blocking hyperactive muscles may prove useful in asymmetry corrections, for example for smile reconstruction in patient with unilateral facial paralysis. Nerve or artery entrapment due to hypertrophic muscle compression may be corrected using BoNT therapy, prior to surgical release of the artery or transposition of the nerve [23,54].

Experimental studies have shown that BoNT therapy is useful in order to improve vascularization of flaps. Protection from primary or secondary ischemia may be possible due to the vasodilation with increased blood flow, therefore reducing peripheral resistance and creating a more robust circulation. In addition to this effect, the blunt response to chemical and cold vasoconstrictive stress of the veins proves to be beneficial in secondary ischemia [23,55]. Based on experimental evidence, clinical trials are required to establish a correct protocol in order to improve survival of free-transferred flaps, especially in patient with vascular comorbidities.

By means of improving vascularization or re-vascularization, according to different studies, the combination between BoNT therapy and adipose tissue engraftment improves the survival of transplanted tissue and reduces the absorption rates. In fact, besides muscle relaxation and favoring settlement of the adipose tissue, the BoNT therapy determines increased levels of CD31 and vascular endothelial growth factor, therefore promoting vasodilation and proliferation of endothelial cells [23,56]. It has no negative effects on adipose-derived stem cells, on the contrary, BoNT promotes adipogenesis through this cells [56]. There are for certain other combination between surgical procedures and BoNT guided administration in order to improve outcomes in the field of plastic surgery and reconstructive microsurgery yet to be discovered.

Pain Management

According to FDA (Food and Drug Administration) type A1 BoNT therapy is only approved for chronic migraine. The other conditions in which this kind of treatment may provide with good outcomes are currently considered off label, even if there are prospective studies that reveal the efficacy of BoNT therapy for different pain conditions. At first, it was believed that the only effect of this treatment was muscle relaxation, therefore, reducing the pain associated with muscle overcontraction and contractures. However, it has been observed that BoNT therapy associated analgesia installs before muscle paralysis, supporting the fact that this treatment modulates the nociceptor system [57]. The neuropeptides and inflammatory substances are blocked, the pain sensors exposure of the plasma membrane is inhibited, therefore, reducing the peripheral sensitization and afferent transmission to the spinal cord and central neurons. Experimental studies have proved the presence of BoNT type A1 specific enzymatic activity in central neurons, following peripheral administration, therefore, suggesting a retro- and anterograde axonal transport of BoNT [58]. In addition, it levels up the antinoceptive factors such as IL-10/IL-1RA in the spine and levels down the pronociceptive factors such as IL-1β/IL-18 [59].

The analgesic effect was first observed in 1985 in treating cervical dystonia using BoNT type A1 therapy [60]. Afterwards, this treatment was used to relieve pain in various conditions such as bruxism, lower back pain, prostatic pain, temporomandibular disorder, facial pain syndrome, various neuropathic pain syndromes and other spastic or non-spastic muscle disorders [61]. According to recent clinical data regarding neuropathic pain, BoNT therapy proved efficient mainly in postherpetic neuralgia,

Table 1

CLINICAL APPLICATION, PRODUCTS AND DOSE REGIMENS

Clinical Application	Product and Dose Regimen	Reference
Blepharospasm	[1)]Botox / [2)]Xeomin 30-60 U [3)]Dysport 60-180 U [4)]Myobloc / [5)]Neurobloc 1200-3600 U	[24] HALLET, M., et al., 2013
Cervical Dystonia	Botox/Xeomin 100-300 U Dysport 400-800 U Myobloc/Neurobloc 10000-20000 U	[24] HALLET, M., et al., 2013
Spasticity for the arm or leg	Botox/Xeomin 300-500 U Dysport 600-1000 U	[24] HALLET, M., et al., 2013
Celiac Plexus Block in Drug-Resistant Hypertension	Botox 100U	[32] LEE, S.H., et al., 2016
Neurogenic Detrusor Overactivity	Botox 200-300 U	[10] GU, H.Y., et al., 2017
Detrusor sphincter dyssynergia **Symptoms of the lower urinary tract associated with benign hyperplasia of the prostate**	Botox 100-300 U	[10] GU, H.Y., et al., 2017
Erectile Dysfunction	Botox 50-100 U	[11] GHANEM, H., et al., 2017
Achalasia	Botox 80-100 U Dysport 240-400 U	[42] LAKE, J.M., Wong, R.K., 2006
Chronic Anal Fissure	Botox 2.5-10 U Dysport 10-40 U	[14] SAHEBALLY, S.M., et al., 2017 [43] BRISINDA, G., et al., 2015
Glabellar lines treatment	Botox 20 U	[45] GUO, Y., et al., 2015
Palmar hyperhidrosis	Botox 75-100 U	[5] DE QUINTANA-SANCHO, A., CONDE CALVO, M.T., 2017
Axillary hyperhidrosis	Botox/Xeomin 50-100 U Dysport 100-200 U Myobloc/Neurobloc 2500 U	[4] NAUMANN, M., et al., 2013
Hypersalivation or sialorrhea	Botox 50 U Dysport 75-450 U Myobloc/Neurobloc 2500 U	[4] NAUMANN, M., et al., 2013

[1)] – onabotulinumtoxin A by Allergan in United States of America; [2)] – IncobotulinumtoxinA by Merz Pharmaceuticals in Germany; [3)] – abobotulinumtoxin A by Ipsen in United Kingdom; [4)] – rimabotulinumtoxin B by Solstice Neuroscience in USA; [5)] – the European version of rimabotulinumtoxin B – Myobloc).

painful diabetic neuropathy and posttraumatic neuralgia [61]. In addition, MORRA, M.E., et al. stated that BoNT therapy may represent an alternative to surgery in treating trigeminal neuralgia [62]. Besides chronic migraine, this kind of therapy was used in order to treat chronic daily headache, episodic migraines and tension related headache. However, according to a meta-analysis performed by JACKSON, J.L., et al., the BoNT therapy may increase the number of days without headaches in chronic daily headache and chronic migraines, but was not as beneficial as expected in tension related headache and episodic migraine [63]. Even though a lot of research has been made regarding the role of this therapy in pain relief, the mechanism is not completely elucidated.

Conclusions

In this review it has been underlined the fact that BoNT is currently used for a wide range of diseases as a symptomatic or etiological treatment, making it a versatile and very powerful instrument. Being able to target so many types of human cells, this therapy is considered of great importance in clinical practice. There are different reports on dose regimens and injection techniques, but from a clinical point a view, standard protocols should be developed for each clinical application, in order to improve medical practice. Therefore, in this paper we presented the most relevant dosage in different pathologies in order to come in hand for medical practice. Even if important discoveries have been made according to recent experimental and clinical studies, there is still much to learn about the therapeutic action of this drug in terms of molecular and pathophysiological mechanisms.

References

1.ZHANG, S., BERNTSSON, R.P., TEPP, W.H., TAO, L., JOHNSON, E.A., STENMARK, P., DONG, M., Nat. Commun., **8**, nr. 1, 2017, p. 1637
2.RUMMEL, A., Toxicon, **107**, nr. Pt A, 2015, p. 9
3.SINGH, S., ALI, M.J., PAULSEN, F., Int. Ophthalmol., [Epub ahead of print], 2017
4.NAUMANN, M., DRESSLER, D., HALLETT, M., JANKOVIC, J., SCHIAVO, G., SEGAL, K.R., TRUONG, D., Toxicon, **67**, 2013, p. 141
5.DE QUINTANA-SANCHO, A., CONDE CALVO, M.T., Actas Dermosifiliogr., **108**, nr. 10, 2017, p. 947
6.CAMPANATI, A., MARTINA, E., GIULIODORI, K., CONSALES, V., BOBYR, I., OFFIDANI, A., Skin Appendage Disord., **3**, nr. 1, 2017, p. 39
7.CHECHERIÞÃ, I.A., SMARANDACHE, D., DAVID, C., CIOCÂLTEU, A., ION, D.A., LASCAR, I., Rom. J. Morphol. Embryol., **53**, nr. 1, 2012, p. 7
8.DAVID, C., PERIDE, I., NICULAE, A., CONSTANTIN, A.M., CHECHERITA, I.A., B.M.C. Nephrol., **17**, nr. 1, 2016, p. 131
9.CHECHERIÞÃ, I.A., DAVID, C., STOICA, L., POPESCU, P., CIOCÂLTEU, A., LASCĂR, I., Rom. J. Morphol. Embryol., **52**, nr. 2, 2011, p. 533
10.GU, H.Y., SONG, J.K., ZHANG, W.J., XIE, J., YAO, Q.S., ZENG, W.J., ZHANG, C., NIU, Y.M., Oncotarget., **8**, nr. 52, 2017, p. 90338
11.GHANEM, H., RAHEEM, A.A., ABDELRAHMAN, I.F.S., JOHNSON, M., ABDEL-RAHEEM, T., Sex Med. Rev., 2017, pii: S2050-0521(17)30085-9
12.MOTEGI, S.I., SEKIGUCHI, A., SAITO, S., ISHIBUCHI, H., KISHI, C., YASUDA, M., ISHIKAWA, O., J. Dermatol., 2017, [Epub ahead of print]
13.ELSHAKH, H., EL-EJJI, K., TAHERI, S., Obes. Surg., **27**, nr. 9, 2017, p. 2471
14.SAHEBALLY, S.M., MESHKAT, B., WALSH, S.R., BEDDY, D., Colorectal Dis., 2017, [Epub ahead of print]
15.GHEORGHIU, M.L., HORTOPAN, D., DUMITRASCU, A., CARAGHEORGHEOPOL, A., STEFANESCU, A., TRIFANESCU, R., NICULESCU, D.A., BACIU, I., CARSOTE, M., POIANA, C., BADIU, C., COCULESCU, M., Acta Endo. (Buc.), **5**, nr. 3, 2009, p. 371
16.LEGGETT, J., HARPER, E., WAITE, E., MARKS, P., MARTINEZ, A., LIGHTMAN, S., Endocrinology, **154**, nr. 9, 2013, p. 3305
17.POIANA, C., NEAMTU, M.C., AVRAMESCU, E.T., CARSOTE, M., TRIFĂNESCU, R., TERZEA, D., NEAMTU, O.M., FERECHIDE, D., DĂNCIULESCU MIULESCU, R., Rom. J. Morphol. Embryol., **54**, nr. 3 Suppl, 2013, p. 717
18.CAPATINA, C., CARAGHEORGHEOPOL, A., BERTEANU, M., POIANA, C., Exp. Clin. Endocrinol. Diabetes, **124**, nr. 8, 2016, p. 461
19.JANKOVIC, J., Mov. Disord., **32**, nr. 8, 2017, p. 1131
20.SUN, S., SURESH, S., LIU, H., TEPP, W.H., JOHNSON, E.A., EDWARDSON, J.M., CHAPMAN, E.R., Cell Host Microbe, **10**, nr. 3, 2011, p. 237
21.FISCHER, A., MONTAL, M., J. Biol. Chem., **282**, nr. 40, 2007, p. 29604
22.MONTECUCCO, C., SCHIAVO, G., PANTANO, S., Trends Biochem. Sci., **30**, nr. 7, 2005, p. 367
23.GRANDO, S.A., ZACHARY, C.B., Br. J. Dermatol., 2017, [Epub ahead of print]
24.HALLETT, M., ALBANESE, A., DRESSLER, D., SEGAL, K.R., SIMPSON, D.M., TRUONG, D., JANKOVIC, J., Toxicon, **67**, 2013, p. 94
25.PICELLI, A., BARICICH, A., CHEMELLO, E., SMANIA, N., CISARI, C., GANDOLFI, M., CINONE, N., RANIERI, M., SANTAMATO, A., Toxins, **9**, nr. 11, 2017, pii: E375
26.AZIZ, J., AWAL, D., AYLIFFE, P., Br. J. Oral Maxillofac. Surg., **55**, nr. 9, 2017, p. 987
27.NEAGU, T.P., ÞIGLIº, M., COCOLOº, I., JECAN, C.R., Rom. J. Morphol. Embryol., **57**, nr. 4, 2016, p. 1215
28.NEAGU, T.P., ÞIGLIº, M., POPP, C.G., JECAN, CR., Rom. J. Morphol. Embryol., **57**, nr. 3, 2016, p. 1051
29.NEAGU, T.P., ENACHE, V., COCOLOº, I., ÞIGLIº, M., COBILINSCHI, C., TINCU, R., Rom. J. Morphol. Embryol., **57**, nr. 2, 2016, p. 437
30.ESQUENAZI, A., ALBANESE, A., CHANCELLOR, M.B., ELOVIC, E., SEGAL, K.R., SIMPSON, D.M., SMITH, C.P., WARD, A.B., Toxicon, **67**, 2013, p. 115
31.WARD, A.B., Eur. J. Neurol., **9**, nr. Suppl 1, 2002, p. 48
32.LEE, S.H., LIM, D.H., LEE, J.H., CHANG, K., KOO, J.M., PARK, H.J., Toxins (Basel), **8**, nr. 2, 2016, 51
33.PERIDE, I., CHECHERITA, I.A., SMARANDACHE, D.R., RADULESCU, D., SINESCU, R.D., NICULAE, A., PRICOP, C., Rom. J. Morphol. Embryol., **56**, 2 Suppl, 2015, p. 777
34.CHECHERITA, I.A., SMARANDACHE, D., RADULESCU, D., PERIDE, I., BRATU, O., CIOCÂLTEU, A., SEBE, I., LASCĂR, I., Chirurgia (Bucur.), **108**, nr. 5, 2013, p. 736
35.WALDFOGEL, J.M., NESBIT, S.A., DY, S.M., SHARMA, R., ZHANG, A., WILSON, L.M., BENNETT, W.L., YEH, H.C., CHELLADURAI, Y., FELDMAN, D., ROBINSON, K.A., Neurology, **88**, nr. 20, 2017, p. 1958
36.ISVORANU, I., PERIDE, I., RADULESCU, D., NICULAE, A., SINESCU, R.D., CHECHERITA, I.A., Rev. Chim.(Bucharest.), **66**, no. 9, 2015, p. 1316
37.THURET, R., HUREL, S., KLEINCLAUSS, F., TIMSIT, M.O., Prog. Urol., **26**, nr. 15, 2016, p. 1114
38.CAPATINA, C., GHINEA, A., DUMITRASCU, A., POIANA, C., Int. J. Diabetes Dev. Ctries., **36**, 2016, p. 393
39.PRICOP, C., BRANISTEANU, D.D., ORSOLYA, M., PUIA, D., MATEI, A., CHECHERITA, I.A., Int. Urol. Nephrol., **48**, nr. 2, 2016, p. 183
40.CHANCELLOR, M.B., FOWLER, C.J., APOSTOLIDIS, A., DE GROAT, W.C., SMITH, C.P., SOMOGYI, G.T., AOKI, K.R., Nat. Clin. Pract. Urol., **5**, nr. 6, 2008, p. 319
41.PLACER, J., SALVADOR, C., PLANAS, J., TRILLA, E., LORENTE, D., CELMA, A., LÓPEZ, M.A., MOROTE, J., J. Endourol., **29**, nr. 3, 2015, p. 332
42.LAKE, J.M., WONG, R.K., Aliment. Pharmacol. Ther., **24**, nr. 6, 2006, p. 909
43.BRISINDA, G., SIVESTRINI, N., BIANCO, G., MARIA, G., Toxins, **7**, nr. 6, 2015, p. 1882
44.COSKUN, H., DURAN, Y., DILEGE, E., MIHMANLI, M., SEYMEN, H., DEMIRKOL, M.O., Obes. Surg., **15**, nr. 8, 2005, p. 1137
45.GUO, Y., LU, Y., LIU, T., ZHOU, Y., YANG, P., ZHU, J., CHEN, L., YANG, Q., Plast. Reconstr. Surg., **136**, nr. 3, 2015, p. 310e
46.DRESSLER, D., Curr. Opin. Microbiol., **15**, nr. 3, 2012, p. 325

47.MAGID, M., FINZI, E., KRUGER, T.H.C., ROBERTSON, H.T., KEELING, B.H., JUNG, S., REICHENBERG, J.S., ROSENTHAL, N.E., WOLLMER, M.A., Pharmacopsychiatry, **48**, nr. 6, 2015, p. 205
48.NAIK, H.B., STEINBERG, S.M., MIDDELTON, L.A., HEWITT, S.M., ZUO, R.C., LINEHAN, W.M., KONG, H.H., COWEN, E.W., J.A.M.A. Dermatol., **151**, nr. 10, 2015, p. 1096
49.NEAGU, T.P., TIGLIS, M., BOTEZATU, D., ENACHE, V., COBILINSCHI, C.O., VALCEA-PRECUP, M.S., GRINESCU, I.M., Rom. J. Morphol. Embryol., **58**, nr. 1, 2017, p. 33
50.NEAGU, T.P., SINESCU, R.D., ENACHE, V., ACHIM, S.C., TIGLIS, M., MIREA, L.E., Rom. J. Morphol. Embryol., **58**, nr. 2, 2017, p. 603
51.HECKMANN, M., HEYER, G., BRUNNER, B., PLEWIG, G., J. Am. Acad. Dermatol., **46**, nr. 4, 2002, p. 617
52.RADULESCU, M., ANDRONESCU, E., CIRJA, A., HOLBAN, A.M., MOGOANTA, L., BALSEANU, T.A., CATALIN, B., NEAGU, T.P., LASCAR, I., FLOREA, D.A., GRUMEZESCU, A.M., CIUBUCA, B., LAZAR, V., CHIFIRIUC, M.C., BOLOCAN, A., Rom. J. Morphol. Embryol., **57**, nr. 1, 2016, p. 107
53.GAZERANI, P., PEDERSEN, N.S., DREWES, A.M., ARENDT NIELSEN, L., Br. J. Dermatol., **161**, nr. 4, 2009, p. 737
54.MURPHY, M., CHARLESWORTH, J., KOH, E., Phys. Ther. Sport, **27**, 2017, p. 7
55.HUANG, L., Br. J. Oral Maxillofac. Surg., 2017, pii: S0266-4356(17)30138-9
56.TANG, Q., CHEN, C., WANG, X., LI, W., ZHANG, Y., WANG, M., JING, W., WANG, H., GUO, W., TIAN, W., Int. J. Mol. Med., **40**, nr. 3, 2017, p. 713
57.PELLETT, S., TEPP, W.H., SCHERF, J.M., PIER, C.L., JOHNSON, E.A., Toxicon, **101**, 2015, p. 63
58.MAZZOCCHIO, R., CALEO, M., Neuroscientist, **21**, nr. 1, 2015, p. 44
59.ZYCHOWSKA, M., ROJEWSKA, E., MAKUCH, W., LUVISETTO, S., PAVONE, F., MARINELLI, S., PRZEWLOCKA, B., MIKA, J., Eur. J. Pharmacol., **791**, 2016, p. 377
60.TSUI, J.K., EISEN, A., MAK, E., CARRUTHERS, J., SCOTT, A.B., CALNE, D.B., Can. J. Neurol. Sci., **12**, nr. 4, 1985, p. 314
61.WHEELER, A., SMITH, H.S., Toxicology, **306**, 2013, p. 124
62.MORRA, M.E., ELGEBALY, A., ELMARAEZY, A., KHALIL, A.M., ALTIBI, A.M., VU, T.L., MOSTAFA, M.R., HUY, N.T., HIRAYAMA, K., J. Headache Pain, **17**, nr. 1, 2016, p. 63
63.JACKSON, J.L., KURIYAMA, A., HAYASHINO, Y., J.A.M.A., **307**, nr. 16, 2012, p. 1736

Manuscript received: 17.07.2017