Toxicon 143 (2018) 90–95

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Globular chitosan prolongs the effective duration time and decreases the acute toxicity of botulinum neurotoxin after intramuscular injection in rats

David S. Sergeevichev [a, *, 1], Anna A. Krasilnikova [a, 1], Artem G. Strelnikov [a], Vladislav V. Fomenko [a, b], Nariman F. Salakhutdinov [a, b], Alexander B. Romanov [a], Alexander M. Karaskov [a], Evgeny A. Pokushalov [a], Jonathan S. Steinberg [c]

[a] "E. Meshalkin National Medical Research Center" of the Ministry of Health of the Russian Federation, Novosibirsk, Russian Federation
[b] Vorozhtsov Institute of Organic Chemistry SB RAS, Novosibirsk, Russian Federation
[c] Department of Medicine, University of Rochester Medical Center, University of Rochester, Rochester, NY, USA

ARTICLE INFO

Article history:
Received 27 November 2017
Accepted 21 January 2018
Available online 31 January 2018

Keywords:
Botulinum toxin A1
Chitosan
Glycosaminoglycans
Threshold stimulation intensity
Block of neuromuscular transmission

ABSTRACT

Botulinum neurotoxin (BoNT) is used for an increasing number of neurological and non-neurological indications and disorders. Since the duration of action of this neurotoxin is limited, the goal of the work was to improve the pharmacological time course of BoNT. We explored the effect of several polysaccharides on the duration of action of BoNT/A1 in rat electromyography. The formulation of BoNT/A1 containing globular chitosan increased the threshold stimulation intensity almost 2 times in 30 days after injection if compared with the baseline threshold. However, conventional linear chitosan, heparin and hyaluronic acid did not have such an effect. In addition, we compared the effectiveness of different doses of BoNT/A1 (25, 50, 75, and 100 U) with globular chitosan and compared the acute toxicity of this formulation with that of BoNT/A1 in physiological saline after intramuscular injection. The results demonstrated that the dose 25 U of BoNT/A1 with globular chitosan was both effective and safe for animals after intramuscular injection. The assessed median lethal dose (LD_{50}) for intramuscular injection in rats was 1.4 times higher for a combination of BoNT/A1 with globular chitosan than that for a solution of BoNT/A1 in physiological saline. Thus, the results of our study have provided evidence that intramuscular injection of the formulation of BoNT/A1 (25 U) containing globular chitosan in rats is safe and significantly prolongs the effective duration time of BoNT/A1.

1. Introduction

Botulinum neurotoxin (BoNT) has become a powerful therapeutic tool in different medical specialties including neurological, ophthalmological, gastroenterological, urological, orthopedic, dermatological, cardiovascular, and cosmetic disorders (Dashtipour and Pedouim, 2016; Dressler, 2012; Jankovic, 2004; Pickett, 2014; Pokushalov et al., 2015; Wheeler and Smith, 2013). Among BoNTs, type A1 botulinum toxin (BoNT/A1) is well established and licensed for clinical use.

BoNT acts on neuromuscular junctions and inhibits the release of acetylcholine from presynaptic membranes, inducing muscle relaxation (Schiavo and Montecucco, 2008). The duration of BoNT activity is an important issue with respect to its clinical use. On the therapeutic side, long lasting BoNT requires fewer injections and lower doses, limiting the possibility of immunization (Pirazzini et al., 2017; Stone et al., 2011). Therefore, it is important to find a way to prolong the therapeutic effect of BoNT.

Generally, the conjugation of a therapeutic protein to polymers or the incorporation of it into a drug carrier for protection and slow release are commonly utilized methods of prolonging the in vivo half-life of proteins (Roberts et al., 2012). In the present study, we investigated if the effective duration time of BoNT/A1 could be prolonged by naturally occurring and biocompatible polysaccharides such as hyaluronic acid, heparin, and chitosan.

* Corresponding author. 15 Rechkunovskaya Str., 630055 Novosibirsk, Russian Federation.
E-mail address: d_sergeevichev@meshalkin.ru (D.S. Sergeevichev).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.toxicon.2018.01.013
0041-0101/

Hyaluronic acid (HA) is universally present in all living organisms as a part of the extracellular matrix. In the literature, a combination of HA fillers and BoNT/A is commonly studied, and improved clinical results have been reported (Carruthers et al., 2003; Kenner, 2011; Küçüker et al., 2016). Heparin, the most negatively charged biopolymer, can interact with a wide range of proteins, with interactions that exhibit a range of specificities and induce several associated biological activities (Cassinelli and Naggi, 2016). Chitosan is a natural, non-toxic, biodegradable and biocompatible polymer; it is quite applicable in tissue engineering and controlled drug release (Duttagupta et al., 2015). It is worth noting that we used two chitosans: conventional linear chitosan (Chitosan) and globular chitosan (Chitozol). A compact globular form of chitosan macromolecules in Chitozol provide better water solubility than that of linear chitosan (Krasilnikova et al., 2018). Therefore, we supposed that these two types of chitosan may have a different effect on the prolongation of BoNT/A1 action.

Therefore, this study was aimed to investigate if these polysaccharides increased the pharmacological activity of BoNT/A1 and prolonged the block of neuromuscular transmission after intramuscular injection. In addition, we compared the effectiveness of different doses of BoNT/A1 (25, 50, 75, and 100 U) in combination with the most effective polysaccharide. Also, we assessed the acute toxicity after intramuscular injection of BoNT/A1 in physiological saline with that of BoNT/A1 in combination with the optimal tested agent.

2. Materials and methods

2.1. Test substances

BoNT/A1 (Xeomin) was purchased from Merz Pharmaceutical GmbH (Germany), and each vial contained a nominal target of 100 U BoNT/A1.

2.1.1. Polysaccharides

Chitosan (conventional linear chitosan macromolecules) was manufactured by Bioprogress (Russian Federation), average molecular weight 450–500 kDa, and the degree of deacetylation 90%.

Chitozol (globular chitosan) was manufactured by Bioavanta (Russian Federation). Average molecular weight of this chitosan was 450–500 kDa, and the degree of deacetylation was at least 90%. A compact globular form of these chitosan macromolecules provides better water solubility than that of conventional linear chitosan.

Chitosan and Chitozol stock solutions were prepared in the equal concentration 1.5%. To prepare 100 mL of 1.5% solution, 0.54 g of succinic acid and 0.45 g of sodium chloride (both Sigma, USA) were dissolved in 100 mL of distilled water under heating and continuous stirring. After complete dissolution of the acid and the salt, 1.5 g of either Chitosan or Chitozol was introduced in small portions; every added portion was completely dissolved. Heating is carried out for 1 h in a microwave oven in the pulsed mode (1/6 time at 600 W). To exclude formation of agglomerates the solutions were treated by ultrasound for 10 min. It must be noted that the Chitosan solution was much more viscous than the Chitozol solution at an equal concentration.

Heparin (Synthesis, Russian Federation), had a concentration 5000 IU/mL. One IU of Heparin is equal to 7.7 μg, so the concentration was 3.85% (38.5 g/L).

Hyaluronic acid (DSM Nutritional Products Europe Ltd., Switzerland), molecular weight 1.45–1.77 MDa, and the concentration of the solution was 1% (10 g/L).

Our preliminary study demonstrated that these polysaccharides did not affect the threshold stimulation intensity and did not have toxic effects after intramuscular injection in investigated doses.

2.2. Animals

Wistar male rats weighing 380–450 g were randomly assigned to experimental groups (n = 10) after 1 week of their adaptations. Rats were housed in a vivarium; commercial laboratory complete food and water were provided ad libitum. The use of rats in this study was approved by Local Ethics Committee of «E. Meshalkin National medical research center» of the Ministry of Health of the Russian Federation (the approval code is 46; the date of approval is 6 March 2015). All parts of the protocol were performed in accordance with recommendations for proper use and care of laboratory animals (European Communities Council Directive 86/609/CEE) and principles of the Declaration of Helsinki.

2.3. Anesthesia

To induce anesthesia, the rats were placed in an anesthesia induction chamber with a continuous supply of air containing sevoflurane 5% (Rodent Ventilator device, Ugo Basile, Italy). Once an animal was anesthetized, it was placed on the operating table, where a 24G peripheral intravenous catheter was inserted into the tail vein. Anesthesia was maintained with intravenous administration of 33 mg/kg sodium thiopental solution every 15–20 min.

2.4. Duration of action of BoNT/A1 in combination with chitosans and GAGs

We investigated four combinations of BoNT/A1 (in the equal concentration) with different polysaccharides and a solution of BoNT/A1 in physiological saline as a control. Groups were as follows:

I—BoNT/A1 (10 U) with physiological saline (0.9%, 500 μL);
II—BoNT/A1 (10 U) with Chitosan (1.5%, 500 μL);
III—BoNT/A1 (10 U) with Chitozol (1.5%, 500 μL);
IV—BoNT/A1 (10 U) with Heparin (3.85%, 500 μL);
V—BoNT/A1 (10 U) with HA (1%, 500 μL).

The combinations I–V (500 μL) were injected in right biceps femoris muscle (BFM) of rats. The contralateral control BFM received the same volume of sterile physiological saline (0.9%).

To compare the denervation effect of BoNT/A1 in combination with different polysaccharides four time points were examined (before injection (b.i.), days 7, 15, and 30 post injection (p.i.)). The time point of injection was considered day 0. At these time points, animals were anesthetized as described previously, a pair of stainless steel stimulating electrodes were inserted into the BFM and an individual muscle contractile response was elicited by applying single rectangular impulses (0.5 ms pulse width). Electrical stimuli were delivered by the pacing system analyzer ERA 300 (Biotronik, USA). The electrical intensity was initially set at 0.5 V and increased in stepwise increments of 0.1 V until the muscle contraction was observed. This minimum electrical intensity that produced visible muscle contraction was registered as the threshold stimulation intensity.

The animals were regularly observed for clinical signs and mortality throughout the experiment.

2.5. BoNT/A1 escalation dose experiment

To define the most effective dose of BoNT/A1 in combination with Chitozol we investigated the following formulations: