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Van Uhm J[1], van der Laarse W[1], Frantzen J[1], Geldof A[1], Beckers G[1], Meuleman E[1]
1. *VU University Medical Center*

AN IN VITRO MODEL TO MEASURE THE BIOACTIVITY OF BOTULINUM NEUROTOXIN TYPE A.

Hypothesis / aims of study

Intravesical botulinum neurotoxin type A (BTX-A) is an effective therapy for patients with refractory overactive bladder (OAB) symptoms (1). The inhibitory effect is considered mainly due to inhibition of acetylcholine release by efferent nerves. However other target sites, like afferent nerves and various receptors in urothelium and suburothelium, also have been reported (2). Still, little is known about translocation and degradation of BTX-A after intravesical injection. In order to improve our knowledge about translocation and degradation BTX-A might be labeled with a radiopharmacon. Radiopharmacon labeled BTX-A enables to identify the biodistribution of BTX-A after intravesical injection. However, the bioactivity of radiopharmacon labeled - and unlabeled BTX-A should be similar. In the present study, we therefore established and validated an in vitro model to measure inhibitory effects of BTX-A on bladder strip contractions.

Study design, materials and methods

Longitudinal bladder strips were obtained from adult male Wistar rats and the urothelium was removed. The strips were mounted in organ baths and perfused by Krebs's solution at 35°C. The strips stabilized for 1 hour adjusting the tension at 10 mN. The organ baths were perfused with KCl solution (80mM) to determine the viability of the strips at the start of the experiment. Carbachol solution (1µM) was used to determine the contractility of the strips before and after BTX-A incubation. The contractility is defined as: PF after BTX-A incubation / PF before BTX-A incubation, where PF = mean of three peak forces of strip contraction induced by carbachol. The strips were stimulated by electric field stimulation (EFS: 100 shocks, 50V, 20Hz, every 3 minutes) during incubation with BTX-A solution (25 and 50 ng/mL) or Krebs' solution (control). The measured inhibition of contraction of bladder strips is defined as: 1 – (PF incubation / PF max) , where PF incubation= mean of five repetitive peak forces of strip contractions induced by EFS every 30 minutes during incubation and, PF max= mean of five highest repetitive peak forces of strip contractions induced by EFS at the start of incubation. Inhibition of strip contraction by BTX-A incubation was described by a log-logistic model: $I = Imax / (1 + \exp(-b * \ln(t / m)))$, where I = measured inhibition of contraction of bladder strips, I_{max} = estimated maximum of inhibition, b = shape parameter, t = time after highest peak forces of EFS at start of incubation (hours) , and m = mid inhibition time, at which $I = 0.5\ I_{max}$ (hours).

Results

All strips were viable at start of the runs. The strips had a contractility of ~ 1 during all runs. BTX-A inhibited EFS induced bladder strip contractions and no effects were seen in controls (figure 1). Various concentrations BTX-A (25 and 50 ng/mL) induced inhibition of bladder strip contraction with a relatively high goodness of fit of the curve (table 1).

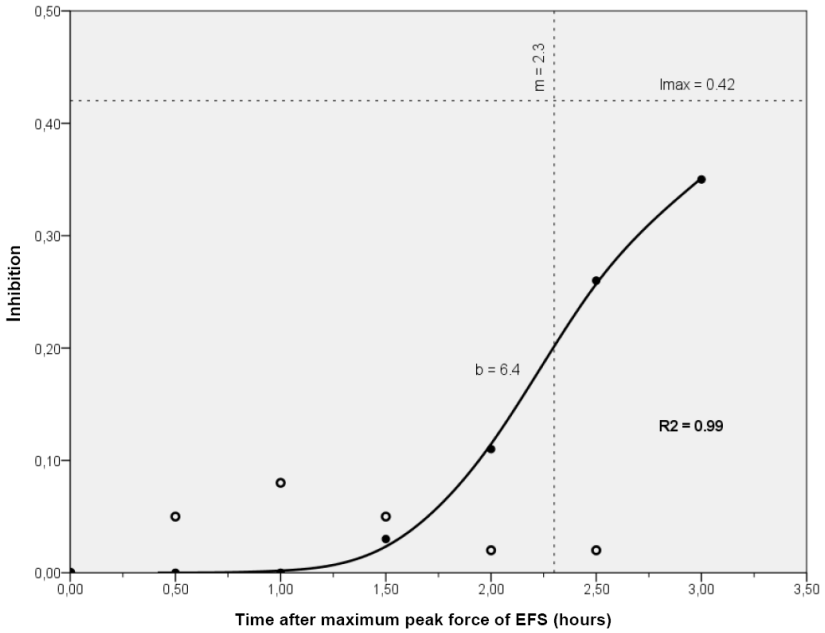

Figure 1: Inhibition of bladder strip contractions by EFS during run 1. The black dots (●) are the measured inhibition of bladder strip contraction after BTX-A incubation (50 ng/ mL) in time (hours) and the fitted curve. The white dots (o) are the measured inhibition of bladder strip contraction after Krebs incubation in time (hours). No log-logistic curve could fit these data indicating lack of inhibition by controls.

BTX-A concentration (ng/mL)	run	Imax	SE	m	SE	b	SE	R_2
controls	1	-		-		-		
controls	2	-		-		-		
25	3	0.91	0.114	1.9	0.22	2.5	0.35	1.00
25	4	1.00	0.479	3.1	1.65	1.7	0.38	0.99
25	5	1.00	0.483	3.3	1.05	3.0	0.81	0.98
50	1	0.42	0.017	2.3	0.04	6.4	0.40	0.99
50	2	0.66	0.077	1.6	0.15	3.0	0.48	1.00
50	4	1.00	0.119	1.8	0.17	3.3	0.61	0.99

Table 1: The data of run 1 till 5 described by log-logistic model.
Imax = estimated maximum of inhibition, m = mid inhibition time (hours), b= shape parameter, R2 = is goodness of fit of the curve.

Interpretation of results

In our organ bath system BTX-A diffused through the various layers of the bladder strip to reach the nerve end terminals. BTX-A had to be endocytosed by the synaptic membrane. BTX-A blocked the exocytosis of acetylcholine and inhibited the contractions of bladder strips. This sequence of biological processes of diffusion and endocytosis of BTX-A is reflected in the observed inhibition of bladder strip contraction, which is described by the log-logistic model presented. The present method of data analysis, in combination with the quantification of the viability of the strips during all runs resulted in this robust in vitro model to establish the bioactivity of BTX-A.

Concluding message

An in vitro model is established and validated to measure inhibitory effects of BTX-A on bladder strip contractions. This in vitro model might be used to measure the bioactivity of radiopharmacon labeled BTX-A and unlabeled BTX-A.

References

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Disclosures

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