Toxicon 138 (2017) 145–150

Contents lists available at ScienceDirect

Toxicon

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

Heart Rate Variability modifications induced by high doses of incobotulinumtoxinA and onabotulinumtoxinA in hemiplegic chronic stroke patients: A single blind randomized controlled, crossover pilot study

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ARTICLE INFO

Article history:
Received 21 April 2017
Received in revised form
28 August 2017
Accepted 30 August 2017
Available online 4 September 2017

Keywords:
Botulinum toxins
Type A
Stroke
Muscle spasticity
Heart rate

ABSTRACT

Background: Botulinum toxin type A is a valid and safe treatment for focal spasticity, with documented effects on both sympathetic and parasympathetic systems. Heart rate variability can provide detailed information about the control of the autonomic nervous system on cardiovascular activities. Previous studies in literature showed no significant changes in Heart Rate Variability with doses >600 U of incobotulinumtoxinA in chronic post stroke spastic patients; however, at present time, these results have not been confirmed with doses >600 U of onabotulinumtoxinA.
Aim: To evaluate changes in Heart Rate Variability induced by high doses (>600 U) of incobotulinumtoxinA or onabotulinumtoxinA in spastic stroke patients over a 1-year period.
Design: single blind randomized controlled crossover study design.
Setting: Rehabilitation Unit of the University Hospital in Novara.
Population: 10 stroke survivors with spastic hemiplegia (Modified Ashworth Scale $\geq$ 2) were recruited and randomly divided in two groups (A and B).
Methods: In the first part of the study, patients in Group A were injected with incobotulinumtoxinA while patients in Group B with onabotulinumtoxinA; after 6 months, a crossover intervention was performed. All patients were blinded to Botulinum toxin type A type, and performed an ECG registration in the 24 h before injection (t0) and 10 days after treatment (t1), both in the first and in the second part of the study. Functional status was evaluated with Barthel Index, Motricity Index and Functional Ambulation Category scores.
Results: Heart Rate Variability analysis showed no significant changes after each Botulinum toxin type A injection in both groups at any evaluation time. Moreover, no statistically significant differences were found regarding each variable between the two groups.
Conclusions: Our data show that high doses (>600 U) of incobotulinumtoxinA and onabotulinumtoxinA do not influence the cardiovascular activity of the autonomic nervous system in chronic hemiplegic spastic stroke survivors.

1. Introduction

Botulinum toxin type A (BoNT-A) has been considered as an effective and safe treatment for focal spasticity in stroke survivors

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http://dx.doi.org/10.1016/j.toxicon.2017.08.027
0041-0101/

(Baker and Pereira, 2013).

BoNT-A can directly modify the heart function acting on the sympathetic (through the preganglionic sympathetic innervation) and parasympathetic systems (through the vagal nerve) (Girlanda et al., 1992), and at present time there is no general consensus on the maximum dose of BoNT-A in terms of safety and clinical interchangeability among the three commercially approved products (abobotulinumtoxinA, onabotulinumtoxinA, incobotulinumtoxinA). In clinical practice the maximum dose admitted per session is up to 400–600 units (U) both for onabotulinumtoxinA (Francisco, 2004; Brin, 1997) and for incobotulinumtoxinA (Wissel and Kempf, 2012). However, two recent open-label studies suggested the safety of doses up to 840 U of incobotulinumtoxinA (Santamato et al., 2013) and of doses up to 800 U of onabotulinumtoxinA (Baricich et al., 2015) for spasticity treatment in stroke survivors.

Heart Rate Variability (HRV) is a simple and non-invasive electrocardiographic derived measure useful to monitor the control of the autonomic nervous system (ANS) on cardiovascular activities including vagal and sympathetic ones (Task Force, 1996; Akselrod et al., 1981). In consideration of its accessibility and low invasiveness, HRV measurement represents a functional indicator of the ANS activity, being a valid method to study the potential impact of high doses of BoNT-A on the autonomic drive directed to the heart in stroke survivors.

Recently no relevant changes have been showed in the autonomic heart drive measured by HRV with doses >600 U of incobotulinumtoxinA in patients with chronic spasticity (Invernizzi et al., 2015). However, at present time, instrumental data about safety of high doses (>600 U) of onabotulinumtoxinA in chronic stroke survivors are lacking. To our knowledge, only Dressler evaluated in a crossover study the safety of high doses of incobotulinumtoxinA (mean dose 450.5 ± 177.1 U) in patients affected by spasticity and previously treated with onabotulinumtoxinA at same doses, showing no differences between the two products in terms of efficacy and adverse events occurrence, evaluated clinically, but without any functional or instrumental indicator (Dressler, 2009). Similarly, Baricich et al. evaluated the efficacy and safety in terms of adverse events of high doses of onabotulinumtoxinA (up to 800 U with a mean of 676.9 ± 86.3 U) in stroke survivors through a clinical evaluation (Baricich et al., 2015). Lastly, Lee et al. compared the efficacy and safety of incobotulinumtoxinA with onabotulinumtoxinA in treating periocular rhytides and masseteric hypertrophy, showing no differences between the two molecules (Lee et al., 2014); however, the maximum dose used was far lower compared to those normally prescribed to treat post stroke spasticity.

In light of these considerations, the aim of this study was to evaluate changes in HRV induced by high doses (>600 U) of incobotulinumtoxinA or onabotulinumtoxinA in spastic stroke patients over a 1-year period using a crossover study design. Moreover, we assessed the treatment safety by monitoring adverse events occurrence.

2. Materials and methods

2.1. Patients

This work was a randomized, single blind, controlled crossover study. A total of 10 stroke survivors with spastic hemiplegia were consecutively enrolled at the Rehabilitation Unit of the University Hospital in Novara from September 2014 to January 2015. The inclusion criteria were: I) focal spasticity graded ≥2 on Modified Ashworth Scale (Bohannon and Smith, 1987) at upper and lower limb muscles, requiring at least 600 incobotulinumtoxinA units; II) hemiplegia after ischaemic or haemorrhagic stroke documented by CT scan and/or available case history; III) age > 18 years. Exclusion criteria were: I) heart failure with NYHA ≥3; II) previous diagnosis of cardiac arrhythmia; III) concomitant use of beta-blockers; IV) pace-maker implant; V) presence of fixed contractures at BoNT-A target muscles.

2.2. Study design

After the enrollment, patients were allocated to one of the two treatment arms by the use of a randomization scheme generated by software. Patients were then divided into two groups (A and B) and entered the first part of the study. In this first part of the study, patients in group A were injected with incobotulinumtoxinA (NT 201; Xeomin®, Merz Pharmaceuticals GmbH, Frankfurt, Germany) with a dilution of 100 units/2 mL of 0.9% sterile saline, while group B patients were injected with onabotulinumtoxinA (Botox® Allergan, Irvine, CA, USA), 100 MU in 2 ml of 0.9% sterile saline. Patients in both groups were blinded to BoNT-A treatment type and received doses below 12 units/Kg.

After 6 months, the second part of the study started, and each group received the crossover intervention: group A patients were treated with onabotulinumtoxinA (Botox® Allergan, Irvine, CA, USA, 100 MU in 2 ml 0.9%NaCl/H2O), whereas group B patients were treated with incobotulinumtoxinA (NT 201; Xeomin®, Merz Pharmaceuticals GmbH, Frankfurt, Germany with a dilution of 100 units/2 mL of 0.9% sterile saline). The same target muscles and doses of the first part of the study were maintained. Study design is described in Fig. 1.

Patients were blinded to BoNT-A treatment type during the whole study period. Each patient signed an informed consent to the treatment and the evaluations of this study. The local committee of our Hospital approved our protocol, which was conducted according to the principles of the Declaration of Helsinki.

2.3. Assessment

All patients in both groups performed a total of four ECG registrations of 30 min each. In the first part of the study, patients performed the ECG registration in the 24 h before BoNT-A injection (baseline, t0) and 10 days after the treatment (t1); similarly, in the second, crossover, part of the study, they performed the ECG registration 24 h before the new BoNT-A injection and 10 days after the treatment. Each ECG recording was performed as described elsewhere (Invernizzi et al., 2015).

Treatment safety was assessed by monitoring adverse events occurrence of any degree.

Lastly, the following functional measures were recorded at baseline: Barthel Index (Shah et al., 1989), Motricity Index (MI) for upper and lower limbs (Collin and Wade, 1990), Functional ambulation category (FAC) (Collen et al., 1990).

Data obtained from analogic ECG were processed with an A/D converter (micro 1401 CED© Cambridge Electronic Design, Cambridge, UK) and recorded on a PC by means of a data acquisition system (Spike2 v.5, CED) with a sampling rate of 3000 Hz. Guidelines were followed for time recordings, sampling rate and HRV analysis of electrocardiograms (Task Force, 1996). Artifacts and noise regions were removed, and, in case of premature beats, they have been manually corrected. Only the recordings that contained <1% of premature beats were considered.

ECG's have been analysed by means of software called “Kubios HRV analysis”, originally created by Niskanen et al. (2004). In this study we considered both linear and non-linear HRV indexes (Buccelletti et al., 2012). A detailed description of all linear and non-linear HRV indexes used in this study can be found in the

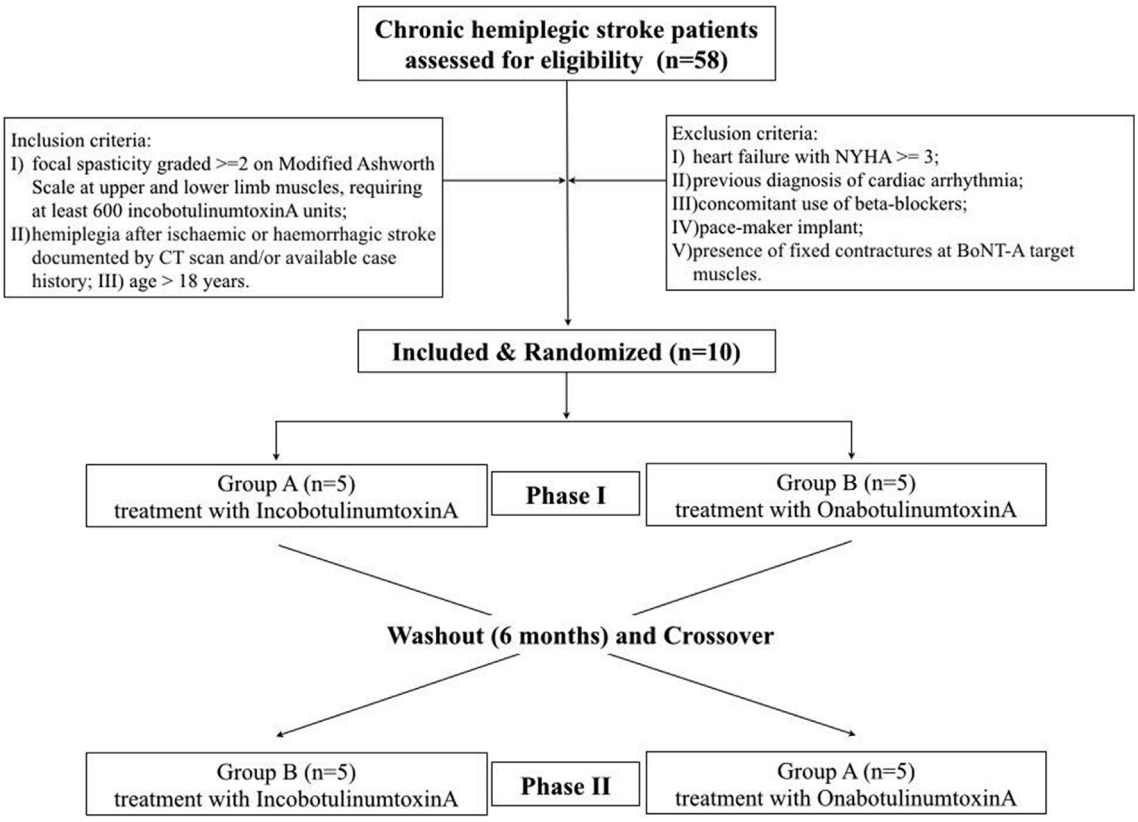

Fig. 1. Study design.

supplementary material of Invernizzi et al. (2015).

2.4. Statistical analysis

Statistical analysis was performed using the GraphPad 6 package, version 6.0 (GraphPad Software, Inc., San Diego, CA, USA). The patients were randomly assigned to one of the treatment arms using a randomization scheme generated by software with a 1:1 allocation and without blocks. Due to the small sample size, we supposed a non-gaussian distribution of variables.

Differences between each variable in each group have been evaluated with Friedman's analysis of variance (ANOVA) and Dunn *post hoc* comparison was used to identify significant differences between mean values. Differences between single variables in different groups were evaluated with the Mann–Whitney U– test. A type I error (alpha) level of 0.05 was chosen.

3. Results

Demographic data of the enrolled patients, dose/kg and mean overall doses of onabotulinumtoxinA and incobotulinumtoxinA utilised are resumed in Table 1. As shown in Tables 2 and 3, none of the variables considered for time, frequency and non-linear domains showed any statistically significant changes after each BoNT-A injection in both Groups A and B at any evaluation time. Moreover, no statistically significant differences were found regarding each variable considered between the two groups. Lastly, one patient in each group experienced mild, self-limiting adverse events after the treatment (pain at the injection site). In each case, adverse events were related to the injection procedure and spontaneously resolved without any intervention 15 min after treatment.

4. Discussion

The results of this study show that high doses (>600 U) of incobotulinumtoxinA and onabotulinumtoxinA do not influence the autonomic drive directed to the heart in chronic hemiplegic spastic stroke survivors. These results are also confirmed if the two BoNT-A formulations are interchanged in a crossover design suggesting the potential absence of cumulative effects. Moreover, both the treatment with incobotulinumtoxinA and the treatment with onabotulinumtoxinA showed only one mild self-limiting adverse event in each group, related to the injective procedure and spontaneously resolved.

The results obtained in this study not only confirm the safety of incobotulinumtoxinA from a cardiovascular point of view, but also, for the first time, produce data about the autonomic heart modifications in stroke survivors with spasticity treated with high doses (>600 U) of onabotulinumtoxinA. In 2009, Dressler in a crossover prospective study converted patients previously treated with onabotulinumtoxinA to incobotulinumtoxinA and repeatedly injected them for 3 years, monitoring clinical efficacy and adverse events occurrence. The author did not find any difference in the efficacy, treatment duration and adverse events occurrence after changing the two drug formulations, suggesting a clinical equivalence of the two BoNT-A formulations (Dressler, 2009). Our results are somehow comparable with those obtained by Dressler, considering also the fact that all outcome measures related to safety in that study were obtained with an anamnestic evaluation only, without any instrumental objective measure. More recently, Mehnert et al. showed similar results observing onabotulinumtoxinA effects on cardiac function after intradetrusor injection, showing no HRV modifications after treatment (Mehnert et al., 2016). The absence of a relevant effect on the autonomic drive

Table 1
Patients' demographical and clinical characteristics. Data are presented as mean ± standard deviation (SD), median and IQR.

	Total	Group A	Group B
Patients (n)	10	5	5
Age (years)	69 ± 10.5	66 ± 14.2	72 ± 5.1
	71 (60.25–76.5)	61 (54–80.5)	73 (67–76.5)
Sex			
Male (n)	7	3	4
Female (n)	3	2	1
Time from stroke (months)	116.8 ± 65.20	126.8 ± 84.95	106.8 ± 49.34
	87 (73.75–161.8)	91.5 (73–215.8)	87 (75.75–157.5)
Type of stroke			
Ischemic (n)	5	3	2
Hemorragic (n)	5	2	3
Weight (kg)	70.3 ± 10.36	73.8 ± 8.42	71.8 ± 9.46
	68 (59–83)	70 (63–78)	71 (62.5–81.5)
BMI	24.37 ± 1.26	25.46 ± 1.38	25.19 ± 1.84
	24.81 (23.88–26.16)	25.24 (24.48–26.37)	25.02 (24.32–26.06)
Total dose of BoNT-A (units)	665 ± 81.82	660 ± 89.44	670 ± 83.67
	625 (600–725)	600 (600–750)	650 (600–750)
Dose pro kg (units)	9.63 ± 0.66	9.48 ± 0.57	9.72 ± 0.61
	9.51 (8.56–10.19)	9.38 (8.66–10.12)	9.55 (8.46–10.27)
Functional Ambulation Category	4.2 ± 0.45	4.2 ± 0.45	4.2 ± 0.45
	4 (4–5)	4 (4–5)	4 (4–5)
Barthel Index	69 ± 6.58	67 ± 5.7	71 ± 7.42
	70 (65–80)	65 (65–75)	70 (70–80)
Motricity Index upper limb	47 ± 3.74	46.2 ± 3.03	47.8 ± 4.59
	47 (47–52)	47 (47–49)	47 (47–52)
Motricity Index lower limb	58 ± 2.58	57.2 ± 3.27	58.8 ± 1.64
	58.5 (57–60)	57 (57–60)	60 (57–60)

Table 2
Results of linear variables of the two groups in the first part of the study and after the crossover procedure, before and after BoNT-A treatment. Mean RR: mean value of RR intervals; Mean HR: mean value of heart rate; RMSSD: root mean square of successive differences; NN50: number of successive intervals differing more than 50 ms; pNN50: relative amount of successive intervals differing more than 50 ms; RR tri index: HRV triangular index; TINN: baseline width of the RR histogram; AR LF: autoregressive modeling based method Low Frequency; AR HF: autoregressive modeling based method High Frequency; AR LF/HF: autoregressive modeling based method Low/High frequency ratio; data are represented as mean and (SD).

	First part				Crossover			
	Group A Mean (SD)	Group B Mean (SD)	Post Group A Mean (SD)	Post Group B Mean (SD)	Group A Mean (SD)	Group B Mean (SD)	Post Group A Mean (SD)	Post Group B Mean (SD)
Mean RR (ms)	807 (79.75)	832 (160.9)	865 (101.5)	885 (108.7)	826 (84.12)	813 (73.2)	859 (88.2)	863 (75.44)
Mean HR (1/min)	75.11 (7.83)	74.18 (13.16)	70.18 (8.15)	69.02 (9.6)	77.3 (6.4)	73.9 (7.4)	72.8 (6.9)	74.5 (8.12)
RMSSD (ms)	29.88 (8.38)	41.15 (18.78)	26.05 (8.78)	41.5 (35.37)	31.45 (7.25)	36.15 (8.42)	33.6 (9.42)	39.7 (7.56)
NN50 (count)	24.5 (14.55)	40.75 (11.59)	22.25 (18.14)	46.1 (24.5)	21.6 (15.29)	32.35 (11.75)	19.8 (13.26)	39.1 (18.01)
pNN50 (%)	3.75 (2.121)	6.03 (2.51)	3.43 (3.3)	6.63 (3.95)	4.8 (2.2)	5.66 (2.48)	4.34 (2.33)	6.63 (2.87)
RR tri index	7.1 (2.66)	7.27 (2.38)	7.16 (0.92)	9.96 (1.39)	8.3 (2.94)	7.87 (2.56)	7.69 (3.47)	6.85 (2.94)
TINN (ms)	241.3 (62.23)	233.8 (38.16)	192.5 (92.42)	242.5 (140.3)	211.6 (58.22)	219.6 (60.73)	187.44 (61.3)	196.2 (54.89)
AR LF (n.u.)	56.4 (29.85)	42.6 (23.87)	56.03 (18.83)	55.35 (19.22)	51.6 (23.13)	50.62 (19.84)	53.4 (20.02)	55.35 (22.66)
AR HF (n.u.)	44.13 (19.27)	57.03 (23.77)	43.85 (18.75)	44.5 (19.2)	48.23 (17.5)	48.84 (15.83)	46.6 (19.43)	44.5 (16.58)
AR LF/HF	2.54 (2.3)	1.22 (1.13)	1.67 (1.3)	1.88 (1.05)	1.91 (1.2)	1.48 (1.07)	1.63 (0.93)	1.83 (1.1)

$p < 0.05$, Post injection Group A vs Group A, first part of the study.
$p < 0.05$, Post injection Group B vs Group B, first part of the study.
$p < 0.05$, Post injection Group B vs Post injection Group A, first part of the study.
$p < 0.05$, Post injection Group A vs Group A, after cross-over procedure.
$p < 0.05$, Post injection Group B vs Group B, after cross-over procedure.
$p < 0.05$, Post injection Group B vs Post injection Group A, after cross-over procedure.

directed to the heart may have a clinical relevance in the treatment decision with high doses of BoNT-A in stroke survivors affected by cardiovascular comorbidities, in which a reduction in HRV is known to be able to increase the incidence of cardiovascular events (Task Force, 1996). Moreover, even in patients without known cardiac diseases, our results can limit the potential concerns about the use of BoNT-A at a dosage greater than 600 units.

As underlined, only few studies in literature investigated the possible autonomic cardiovascular modifications induced by onabotulinumtoxinA, and with doses far lower than those utilised in our work. Lastly, the fact that these two drugs at the same dose in the same patients at different times induce the same modifications on HRV can in part explain the clinical results regarding efficacy and safety found by Dressler (2009). However, due to the low sample size, our data should be taken cautiously and for this reason further experience with high dose use of BoNT-A is needed.

As a last consideration, our data suggest also, in line with the results obtained by Dressler, that the treatment with high doses of BoNT-A (both onabotulinumtoxinA and incobotulinumtoxinA) does not seem to produce any cumulative effect on HRV modifications and adverse events occurrence. Even though the overall study period involved only two BoNT-A injections, these data are consistent with previous studies of safety in multiple spasticity treatment performed with BoNT-A and give further elements about

Table 3
Results of non-linear variables of the two groups in the first part of the study and after the crossover procedure, before and after BoNT-A treatment. Lmean: Recurrence plot analysis - mean line length; Lmax: Recurrence plot analysis - maximum line length; REC: Recurrence plot analysis - recurrence rate; DET: determinism of the time series; ShanEn: Shannon entropy; ApEn: Appoximate Entropy; SampEn: Sample Entropy; DFAa1: Detrended fluctuation analysis - short-term fluctuations; DFAa2 Detrended fluctuation analysis - long-term fluctuations; D2: Correlation dimension; SD1: standard deviation of the Poincaré plot perpendicular to line of identity; SD2: standard deviation of the Poincaré plot along the line of identity; data are represented as mean and (SD).

	First part				Crossover			
	Group A Mean (SD)	Group B Mean (SD)	Post Group A Mean (SD)	Post Group B Mean (SD)	Group A Mean (SD)	Group B Mean (SD)	Post Group A Mean (SD)	Post Group B Mean (SD)
Lmean (beats)	19.62 (10.13)	20.69 (11.26)	14.23 (4.8)	17.83 (6.42)	16.54 (9.42)	21.29 (12.45)	14.87 (8.45)	18.42 (10.48)
Lmax (beats)	180.2 (172.5)	243.1 (142.5)	210.3 (131.8)	237.5 (122.3)	226 (168.2)	218.3 (130.3)	238.36 (137.2)	215.44 (119.76)
REC (%)	38.58 (15.43)	48.17 (10.42)	42.94 (11.36)	44.55 (11.85)	36.1 (18.7)	42.34 (9.66)	43.75 (12.45)	41.15 (13.72)
DET (%)	103.2 (4.9)	98.85 (1.13)	98.77 (2.04)	99.08 (3.13)	144 (8.3)	84.5 (7.47)	106.48 (20.42)	101.38 (18.81)
ShanEn	3.43 (0.67)	3.5 (0.33)	3.4 (0.37)	3.53 (0.36)	3.56 (0.41)	3.3 (0.68)	3.41 (0.74)	3.23 (0.45)
ApEn	1.09 (0.32)	1.01 (0.37)	1.11 (0.19)	0.93 (0.31)	1.02 (0.27)	0.97 (0.19)	1.04 (0.21)	1.02 (0.3)
SampEn	1.84 (0.33)	1.63 (0.31)	1.3 (0.25)	0.99 (0.42)	1.7 (0.42)	1.42 (0.26)	1.34 (0.53)	1.59 (0.61)
DFA:a1	1.132 (0.41)	0.7 (0.38)	0.99 (0.25)	1.02 (0.28)	0.89 (0.33)	0.84 (0.22)	1.043 (0.46)	0.93 (0.29)
DFA:a2	0.933 (0.19)	0.89 (0.3)	0.95 (0.12)	0.98 (0.15)	0.96 (0.26)	0.91 (0.33)	0.94 (0.29)	0.95 (0.41)
D2	0.73 (0.28)	0.58 (0.31)	0.55 (0.1)	0.83 (0.28)	0.64 (0.18)	0.66 (0.27)	0.73 (0.15)	0.71 (0.38)
SD1 (ms)	15.74 (11.51)	29.13 (13.34)	18.43 (6.25)	29.38 (15.02)	17.7 (10.63)	19.32 (9.49)	19.12 (12.36)	18.12 (14.72)
SD2 (ms)	39.16 (18.32)	44.03 (18.47)	39.9 (5.2)	54.60 (29.24)	38.89 (20.71)	45.42 (23.52)	39.07 (25.13)	42.60 (30.26)

$p < 0.05$, Post injection Group A vs Group A, first part of the study.
$p < 0.05$, Post injection Group B vs Group B, first part of the study.
$p < 0.05$, Post injection Group B vs Post injection Group A, first part of the study.
$p < 0.05$, Post injection Group A vs Group A, after cross-over procedure.
$p < 0.05$, Post injection Group B vs Group B, after cross-over procedure.
$p < 0.05$, Post injection Group B vs Post injection Group A, after cross-over procedure.

overall BoNT-A safety at high doses (>600 U) in hemiplegic stroke survivors (Santamato et al., 2013; Baricich et al., 2015).

This study has several limitations: the first is the low sample size; the second is the overall study duration (1 year) that hinders the possibility to evaluate long-term modifications induced by multiple BoNT-A injections.

5. Conclusions

In conclusion, this study confirms the safety of incobotulinumtoxinA at doses up to 12 units/kg regarding the autonomic heart drive modifications and adverse events occurrence in adult stroke hemiplegic spastic patients. Moreover, for the first time in literature, this study shows the same results in terms of safety of onabotulinumtoxinA. Lastly, multiple administrations of BoNT-A at high doses in adult hemiplegic patients do not seem to induce any cumulative effect. However, further experience with high doses of BoNT-A is needed in order to confirm these results and, in particular, for the clinical use of these two BoNT-A formulations in patients with cardiovascular comorbidities.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

Alessio Baricich: personal fees from Allergan, Ipsen, and Merz, outside the submitted work; Elisa Grana: none; Stefano Carda: none; Andrea Santamato: personal fees from Ipsen and Merz, outside the submitted work; Claudio Molinari: none; Carlo Cisari: none; Marco Invernizzi: none.

Ethical statement

All authors certified that have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

Acknowledgements

The authors wish to thank Alessandro de Sire, MD for manuscript revision.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.toxicon.2017.08.027.

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