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Measuring the potency labelling of onabotulinumtoxinA (Botox®) and incobotulinumtoxinA (Xeomin®) in an LD50 assay

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Abstract The biological potency of botulinum toxin (BT) drugs is determined by a standardised LD50 assay. However, the potency labelling varies vary amongst different BT drugs. One reason for this may be differences in the LD50 assays applied. When five unexpired batches of onabotulinumtoxinA (Botox®) and incobotulinumtoxinA (Xeomin®) are compared in the Xeomin® batch release assay, the potency variability of both BT drugs fell within the range allowed by the European Pharmacopoiea. Statistical analyses failed to detect differences in the potency labelling of both products. Although the existence of a conversion ratio has been questioned recently, our experimental data are in line with previous clinical experience showing that Botox® and Xeomin® can be compared using a 1:1 conversion ratio. Identical potency labelling allows easy exchange of both BT drugs in a therapeutic setting, and direct comparison of efficacy, adverse effects and costs.

Keywords Botulinum toxin type A · OnabotulinumtoxinA · IncobotulinumtoxinA · Complexing proteins · LD50 assay · Conversion ratio · Botox® · Xeomin®

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Introduction

Botulinum toxin (BT) drugs have been used for many years in numerous medical specialties. Their safe and efficacious use is based upon a standardised labelling of their biological potency. As described in the European Pharmacapoiea the biological potency of BT drugs is measured by a standardised LD50 assay (European Pharmacopoeia 2008a, b) and expressed in mouse units. The Merz mouse unit (MU) is derived from the Merz LD50 assay, which was originally qualified against a BT type A standard, available from the National Institute for Biological Standards and Control (NIBSC, Potters Bar, Herts, UK). Whereas the consistency of potency measurements is closely monitored for each BT drug by the registration authorities, clinical practise suggests that the potency between the different BT drugs varies considerably. Between the potency labelling of onabotulinumtoxinA (Botox®) and abobotulinumtoxinA (Dysport®) conversion factors from 1:5 to 1:2.41 have been reported in clinical studies (Brin and Blitzer 1993; Marion et al. 1995; Marsden 1993; Van den Bergh and Lison 1996; Ranoux et al. 2002). In LD50 assays conversion factors of 1:2.89 (Hambleton and Pickett 1994), 1:2.86 (Van den Bergh and Lison 1996) and 1:1.9 (First et al. 1994) were determined. Between the potency labelling of Botox® and the potency labelling of rimabotulinumtoxinB (NeuroBloc®/Myobloc®), the conversion ratio seems to be 1:40 (Dressler and Bigalke 2009). The reasons for the incomparability of the potency labelling are unclear. The differences amongst the particular test systems used by each manufacturer may be one of them. We, therefore, sought to test the BT type A drugs Botox® and incobotulinumtoxinA (Xeomin®) (for product comparison see Table 1) in one standardised test system to determine whether their potency labelling is identical.

Table 1 Comparison between the botulinum toxin drugs onabotulinumtoxinA (Botox®) and incobotulinumtoxinA (Xeomin®)

	onabotulinumtoxinA (Botox®)	incobotulinumtoxinA (Xeomin®)
MU per vial	50/100/200	100
Botulinum neurotoxin type	A	A
Manufacturing process	Spray vacuum-drying	Lyophilisation
Molecular composition	900 kDa botulinum toxin complex	150 kDa botulinum neurotoxin
Total clostridial protein content per 100 MU vial	5.0 ng	0.44 ng
Neurotoxin content per 100 MU vial	0.73 ng	0.44 ng
Specific biological potency	137 MU/ng	227 MU/ng
Excipients content per 100 MU vial	0.5 mg human serum albumine 0.9 mg NaCl	1.0 mg human serum albumine 4.7 mg sucrose

Methods

Five batches of Xeomin® (Merz Pharmaceuticals, Frankfurt/M, Germany) (40801, 40802, 80207, 70406, 61102) and five batches of Botox® (Pharm-Allergan, Ettlingen, Germany) (C1513C2, C1534C1, C1385C2, C1643C1, C1641C1) were used in the Xeomin® batch release LD50 assay according to the standard operation procedures to determine the biological potency of both BT drugs. All batches were tested within their specified shelf life (remaining shelf life: Botox® 39%, Xeomin® 60%). The potencies of the batches were measured in two to six single test sessions per batch by injecting the mice intraperitonealy (volume 0.5 ml, needle gauge 27, dilution factor 1.3 between doses) and monitoring the percentage of mortality across dilutions over 72 h. To determine relative potencies of Botox® and Xeomin®, the results were subjected to a quantal response parallel-line probit analysis, and the results of consecutive test sessions were combined. Potency quantification was performed using the Xeomin® reference standard qualified against the NIBSC botulinum toxin type A standard (A/23, # 93/810). The qualification of a reference standard is performed in a consecutive set of experiments employing the identical method, and requires multiple determinations of both the reference standard and the material to be qualified. The mean values of repeat measurements were compared by a two-tailed *t* test for unpaired data.

Results

As shown in Table 2, the biological potency of the Botox® batches studied ranged from 96.6 to 111.0 MU. The difference between the batch with the lowest biological potency and the batch with highest biological potency was 14.4 MU. For Xeomin® the biological potency ranged from 99.0 to 114.6 MU. The difference between the batch with the lowest biological potency and the batch with the highest biological potency was 15.6 MU. The mean and standard deviation for the Botox® and Xeomin® batches tested were 103.1 ± 6.5 and 101.7 ± 6.2 MU, respectively. The two-tailed *t* test did not show a significant difference between the biological potencies of both BT drugs ($p = 0.73$).

Table 2 Measurement of potency labelling of five batches of onabotulinumtoxinA (Botox®) and five batches of incobotulinumtoxinA (Xeomin®) in an LD50 assay

OnabotulinumtoxinA (Botox®)		IncobotulinumtoxinA (Xeomin®)	
Batch	Potency (MU)	Batch	Potency (MU)
C1513C2	111.0	40801	99.0
C1534C1	104.9	40802	100.0
C1385C2	96.6	80207	102.5
C1643C1	97.5	70406	99.6
C1641C1	98.4	61102	114.6
Mean	103.1	Mean	101.7
Standard deviation	6.5	Standard deviation	6.2
Confidence interval upper limit	94.0	Confidence interval upper limit	95.0
Confidence interval lower limit	109.3	Confidence interval lower limit	111.2
n	5	*n*	5
t test, two-tailed, $p = 0.73$			

Discussion

With potency ranges from 96.6 to 111.0 MU for Botox® and 99.0 to 114.6 MU for Xeomin®, the biological potencies of both BT drugs were well within the ranges of 100 MU + 25% and 100 MU − 20%, allowed by the European Pharmacopoeia 6.0 (2008a, b). This production variability marks high production standards, and should be kept in mind when clinical dosing is discussed.

Recently, another LD50 study compared the potency labelling of Botox® and Xeomin® suggesting a conversion ratio of 1:0.8 between Botox® and Xeomin® (Hunt and Clarke 2009a, b). This finding contradicts the 1:1 conversion ratio shown in previous clinical studies on patients with cervical dystonia (Benecke et al. 2005), blepharospasm (Roggenkämper et al. 2006), hyperhidrosis (Dressler 2010) and various other dystonias and spasticity (Dressler 2009). The Reasons for this discrepancy are not given by the authors. Given these unexpected results, we tried to repeat this study using a potency assay used for the official Xeomin® batch release. With mean and standard deviations of 103.1 ± 6.5 MU for Botox® and 101.7 ± 6.2 MU for Xeomin®, there were no statistically significant differences between the potencies of both BT drugs measured in our study (two-tailed t test, $p = 0.73$). The reasons for the diverging results of the Hunt and Clarke study and our study are not clear. They may include methodological differences of the two LD50 assays applied. Whereas we added HSA as additional botulinum neurotoxin protection when it is further diluted for therapeutic purposes (McLellan et al. 1996), this additional protection was not provided in the Hunt and Clarke assay (Mander et al. 2009). Other differences between both assays may exist, but have not been reported by the authors.

Direct comparison between potency labels of different BT drugs bears risks as recognised for years. A recent warning of the US Food and Drug Administration pointed out these risks again. Potency comparison of BT drugs, however, is essential for comparison of efficacy, adverse effects and costs of the growing number of BT drugs entering the market. Although, the existence of a conversion rate has been questioned recently, our experimental data confirm previous clinical data in patients with dystonia, spasticity and hyperhidrosis showing that, Botox® and Xeomin® can be compared by using a 1:1 conversion ratio.

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