Botulinum Toxin

Formulation, Concentration and Treatment

ALMA RYSTEDT
Botulinum toxin (BTX) is used in various fields of medicine, including the treatment of hyperhidrosis and cervical dystonia. Botox®, Dysport®, Xeomin® and NeuroBloc® are commercially available BTX products, which are formulated differently and their dosing units are unique. Dosage and concentration of the prepared solution for injection varies considerably among studies comparing the products. Improved guidelines on concentration and dosing when changing from one product to another are warranted. This would ensure the use of the lowest effective doses for good effect, minimal risk of antibody formation and side-effects as well as reduced costs.

The aim of the present work was to find the most appropriate BTX concentration for each of the four products to achieve the highest sweat reducing effect and to investigate dose conversion ratios between Botox and Dysport in the treatment of cervical dystonia when the products are diluted to the same concentration, 100 U/ml.

Paper I and II clearly confirm that it is crucial to consider the BTX concentration in a treatment regimen, especially when changing between different products. The optimal concentration to reduce sweating varies among the products and was found to be 25 U/ml for Botox and Xeomin, approximately 100 U/ml for Dysport and 50 U/ml for NeuroBloc. However, for NeuroBloc the optimal concentration might be even lower.

In Paper III, which is a retrospective study using casebook notes from 75 patients with cervical dystonia, it was found that the most appropriate dose conversion ratio to use when switching from Botox to Dysport was 1:1.7.

In Paper IV, Botox and Dysport were prospectively compared in a double-blind, randomized clinical trial in two different dose conversion ratios (1:3 and 1:1.7) when diluted to the same concentration (100 U/ml). No statistically significant difference was seen between Botox (1:3) and Dysport nor between Botox (1:1.7) and Dysport four weeks after treatment. Some of the secondary outcome observations, however, did indicate that the ratio 1:3 resulted in suboptimal efficacy of Botox but this must be further validated in a larger patient material.

Keywords: Botulinum toxin, Botox, Dysport, Xeomin, NeuroBloc, Hyperhidrosis, Cervical dystonia

Alma Rystedt, Uppsala University, Department of Neuroscience, Neurology, Akademiska sjukhuset, SE-751 85 Uppsala, Sweden.

© Alma Rystedt 2012

ISSN 1651-6206
ISBN 978-91-554-8481-1
urn:nbn:se:uu:diva-181667 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-181667)
To my parents
Maj & Bernt Karlsson
This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


Reprints were made with permission from the respective publishers.
Book cover: Uppsala Cathedral and Castle by Einar Rystedt.
# Contents

Introduction ................................................................................................... 11

Background ................................................................................................... 12
  Botulinum toxin (BTX) ............................................................................ 12
    History ................................................................................................... 12
    Origin .................................................................................................... 12
    Mechanisms of action .......................................................................... 14
    Termination of BTX action ................................................................. 16
    Sprouting ............................................................................................. 16
    Products ............................................................................................... 16
  Mechanisms of sweating and Hyperhidrosis ............................................ 19
  Cervical dystonia ...................................................................................... 19

Aims .............................................................................................................. 20
  General aim .............................................................................................. 20
  Specific aims ............................................................................................ 20

Material and Methods ................................................................................... 21
  Paper I ...................................................................................................... 21
    Injection procedure .............................................................................. 21
    Iodine-starch test .................................................................................. 21
    Area measuring .................................................................................... 22
    Anhidrotic mean area and anhidrotic mean area per unit .................... 22
    Statistical analyses ............................................................................... 22
  Paper II ..................................................................................................... 22
    Randomization and injection procedure .............................................. 22
    Iodine-starch test .................................................................................. 23
    Area measuring .................................................................................... 23
    Anhidrotic and hypohidrotic mean areas per unit ................................ 23
    Primary objective and endpoint ........................................................... 25
    Secondary objectives ........................................................................... 25
    Statistical analyses ............................................................................... 25
  Paper III .................................................................................................... 25
    Study design ........................................................................................ 25
    First analysis set .................................................................................. 26
    Second analysis set .............................................................................. 27
    Statistics ............................................................................................... 27
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>BTX</td>
<td>Botulinum toxin</td>
</tr>
<tr>
<td>BTX A</td>
<td>Botulinum toxin, serotype A</td>
</tr>
<tr>
<td>BTX B</td>
<td>Botulinum toxin, serotype B</td>
</tr>
<tr>
<td>FDQ</td>
<td>Functional Disability Questionnaire</td>
</tr>
<tr>
<td>MEI</td>
<td>Movement Energy Index</td>
</tr>
<tr>
<td>SF-36</td>
<td>Short Form Health Survey -36</td>
</tr>
<tr>
<td>SNAP-25</td>
<td>Synaptosomal associated protein, molecular weight 25 kDa</td>
</tr>
<tr>
<td>SNARE</td>
<td>Soluble NSF (N-ethylmaleimide sensitive fusion) attachment protein receptor</td>
</tr>
<tr>
<td>TWSTRS</td>
<td>Toronto Western Spasmodic Torticollis Rating Scale</td>
</tr>
<tr>
<td>U</td>
<td>LD&lt;sub&gt;50&lt;/sub&gt;-mouse unit</td>
</tr>
<tr>
<td>VAMP</td>
<td>Vesicle Associated Membrane Protein</td>
</tr>
</tbody>
</table>
Introduction

Botulinum toxin (BTX) is used in various fields of medicine, including the treatment of hyperhidrosis and cervical dystonia. Local injections of BTX prevent release of acetylcholine and co-transmitters in peripheral cholinergic nerve endings, which consequently leads to reduced symptoms.

Botox® (Allergan Inc, Irvine, CA, USA), Dysport® (Ipsen Limited, Slough, UK), Xeomin® (Merz Pharma GmbH & Co. KGaA, Frankfurt/Main, Germany) and NeuroBloc® (Eisai Limited, Hatfield, United Kingdom) are commercially available BTX products in Sweden. The products are formulated differently and their dosing units are unique. Variability in dose-response with respect to these products when treating muscular disorders has been presented in the literature. Botox has been reported to be 3-6 times more potent than Dysport, and NeuroBloc has been used in doses 50-100 times higher than Botox. Xeomin has been used in the same doses as Botox with similar treatment response.

BTX concentration and albumin content in the prepared solution have been suggested to affect the utilization of BTX and consequently the effect. Improved guidelines on concentration and dosing when changing from one product to another are warranted. This would ensure the use of the lowest effective doses for good effect, minimal risk of antibody formation and side-effects as well as reduced costs.

The aim of the present work was to find the most appropriate concentration of Botox, Dysport, Xeomin and NeuroBloc, respectively, to achieve the highest sweat reducing effect and to investigate dose conversion ratios between Botox and Dysport in the treatment of cervical dystonia when the products are diluted to the same BTX concentration, 100 U/ml.
Background

Botulinum toxin (BTX)

History
At the beginning of the 19th century the poet and physician Justinus Kerner published the first accurate description of the clinical symptoms of, what now would be diagnosed, botulism (1). The patients were suffering from, among other things; extreme drying out of the palms and soles, absence of saliva secretion, difficulties in urinating and muscle paralysis. The muscle paralysis could finally lead to respiratory failure. The source of the symptoms was badly prepared sausages. After observing a number of patients with food poisoning Justinus Kerner started to perform experiments on animals using extract from sausages containing the substance he called “sausage poison”. The clinical symptoms were similar to those in humans. Based on his experiences he predicted future use of the toxin, applied in minimal doses, in the treatment of several diseases.

The microbiologist Emile Pierre van Ermengem correlated the “sausage poisoning” with a microorganism he found in ham that had been served to a group of people presenting clinical symptoms of botulism (2). Between 1895-1897 he discovered and isolated the anaerobic bacterium, which produces the “sausage poison”. He called it “Bacillus botulinus” (botulus is the Latin word for sausage), but that was later changed to Clostridium botulinum. The poison is nowadays called Botulinum toxin (BTX).

Origin
Seven immunologically distinct serotypes of BTX have been identified, type A to G (3).

BTX is synthesised as a single chain polypeptide with a molecular weight of approximately 150 kDa (4). Later in the process the single chain is “nicked” into a dichain structure by proteases; this is needed to activate the BTX (4, 5). The native dichain, also known as the derivative toxin, consists of a heavy chain (about 100 kDa) and a light chain (about 50 kDa) which are held together by a disulphide bridge (6).

There are three, distinct, functional domains within the native BTX (7, 8). The carboxy-terminal domain is accountable for neurospecific binding and
the amino-terminal domain is involved in the membrane translocation, both these domains are located on the heavy chain. The NH₂-terminal domain, placed on the light chain, is a zinc endopeptidase responsible for cleaving the enzymes necessary for exocytosis of the neurotransmitter (acetylcholine). Figure 1 shows a three-dimensional model of the native BTX.

![Figure 1](image)

Figure 1. Three-dimensional crystal structure of BTX A. Red and yellow highlighting show the parts of the neurospecific binding domain, the green highlighting shows the membrane translocation domain and the blue highlighting shows the protease domain. (Figure reprinted with permission from the journal; J Physiol Paris. 2002;96:105-13).

The 150 kDa molecule is covered by one or more non-toxic stabilising and protecting proteins that may or may not have haemaglutinin activity (5). The particular composition and molecular weight of the protein complex depends on the serotype and the bacterial strain that produced it. The complexes, termed progenitor toxins, can be classified into different groups based on their molecular sizes; LL (extra large, ~900 kDa), L (large, ~500 kDa) and M (medium, ~300 kDa). In some articles the molecular sizes are named according to their original classification, the sedimentation value, which are 19S (LL), 16S (L) and 12S (M) (6).

Neither of the stabilising and protecting proteins contributes in the BTX-induced blockade of cholinergic transmission (4). The complex dissociates from the 150 kDa dichain in physiological pH (4, 9). These proteins, however, play an important role for oral poisoning since the complex protects the BTX from degradation due to proteolytic enzymes and low pH in the stomach (4). The role of the complex in immunoresistance is obscure but antibodies directed against these accompanying proteins may occur (10).
Mechanisms of action

All BTX serotypes act by preventing acetylcholine release at peripheral nerve endings. This proceeds through a four-step mechanism: binding, internalisation, membrane translocation and enzymatic target modification (8). The mechanisms of action for BTX are shown in Figure 2.

Figure 2. A) Normal neurotransmitter release at a neuromuscular junction. B) Neuromuscular junction exposed to BTX. (Figure reprinted with permission from the journal; JAMA. 2001;285:1059-1070).
The toxins bind to the membranes of cholinergic nerve endings rapidly and with high affinity (7). The binding site on the BTX is, as mentioned earlier, the carboxy-terminal domain on the heavy chain (8). With the exception of serotypes C and D, which are closely related, other toxin serotypes each have their own binding sites that are partially or wholly unique (4). The characterisation of the BTX receptors is not yet complete, but a large number of studies have established that polysialogangliosides are involved in the binding (7, 8). Furthermore it is widely accepted that additional proteins exposed to the cell surface are implicated. Experiments point to an involvement of the synaptic vesicle proteins synaptotagmins I and II in the binding of different serotypes of BTX.

BTX has been found to cross the membrane by endocytosis (7, 8). When the vesicles containing the internalized BTX are inside the neurons, the light chain must cross the hydrophobic vesicle membrane to reach the cytosol where it performs the mode of action (7, 8). For this process the BTX needs to be exposed to a low pH (4.40-4.60) step (11). The low pH induces a conformational change of the BTX, converting it from a water-soluble “neutral” form to an “acid” form with surface-exposed hydrophobic segments (7, 8). This enables the penetration of both the heavy and the light chains in the hydrocarbon core of the lipid bilayer. After the membrane insertion, BTX forms ion channels in the bilayer, which are believed to mediate the process of translocation of the light chain across the vesicle membrane into the neuron cytosol. It is the amino-terminal domain on the heavy chain that is implicated in the membrane translocation.

The various BTX serotypes are specific, zinc-dependent proteases that act by cleaving different intracellular proteins, or cleave the same protein but at distinct sites (5, 7, 8). The NH₂-terminal domain, placed on the light chain, is responsible for the destruction. Three substrate proteins have been identified: vesicle associated membrane protein (VAMP) also called synaptobrevin, synaptosomal associated protein, molecular weight 25 kDa (SNAP-25) and syntaxin. BTX serotypes B, D, F and G cleave, at different sites, VAMP; BTX serotypes A and E cleave SNAP-25 at two different sites and BTX C cleaves both SNAP-25 and syntaxin. The three proteins, which have dissimilar intracellular locations, are associated with synaptic vesicles (VAMP) or with the synaptic membrane (SNAP-25 and syntaxin). Together they form a complex; soluble NSF (N-ethylmaleimide sensitive fusion) attachment protein receptor (SNARE), that mediates the fusion of neurotransmitter-containing vesicles with the synaptic membrane. This interaction ultimately leads to the release of neurotransmitter into the synapse. Destruction of a protein in the SNARE complex consequently inhibits the release of acetylcholine and co-transmitters from the nerve ending.
Termination of BTX action

So far the mechanism that accounts for termination of BTX action is unknown (4). No evidence that the light chain is transported across the plasma membrane to reach the extracellular space has been found, which would suggest loss of activity due to intracellular disposition of the toxin (diffusion away from the nerve ending where the proteolysis takes part) (4). There could also be a metabolic process for degradation of the BTX, most likely with the involvement of endoproteases (4).

Sprouting

A number of studies concerning functionality and paralysis are performed on α-motorneurons with innervated muscles. The nerve endings have not been found to be damaged due to the intoxication; they are however dysfunctional, which triggers extensive sprouting initiated by growth factors from the paralysed muscles (8, 12, 13). The sprouts are processed from the nerve terminals or nodes of Ranvier and have the ability to form functional synapses. When the BTX treated original nerve terminals later recover, the temporary sprouts disappear (13). The duration of BTX effect varies with serotype and it has been indicated that serotype A has the most sustained action (4). Effect duration also depends on individual factors with a large interindividual variation in the treatment of hyperhidrosis (14). It has also been found that sprouting occurs in sudomotorneurons with innervated sweat glands; however, this field is less studied (15).

Products

Botox® (onabotulinumtoxinA), Dysport® (abobotulinumtoxinA), Xeomin® (incobotulinumtoxinA) and NeuroBloc® (rimabotulinumtoxinB) are commercially available BTX products in Sweden. Detailed information about these products are shown in Table 1 (16-23).
<table>
<thead>
<tr>
<th></th>
<th>Botox</th>
<th>Dysport</th>
<th>Xeomin</th>
<th>NeuroBloc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Allergan</td>
<td>Ipsen</td>
<td>Merz</td>
<td>Eisai</td>
</tr>
<tr>
<td>Serotype</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Complex size</td>
<td>900 kDa</td>
<td>–</td>
<td>150 kDa</td>
<td>700 kDa</td>
</tr>
<tr>
<td>Units/vial</td>
<td>100 U&lt;sup&gt;b&lt;/sup&gt;</td>
<td>500 U&lt;sup&gt;c&lt;/sup&gt;</td>
<td>100 U&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5 000 U&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Amount of neurotoxin-protein</td>
<td>5 ng/100 U</td>
<td>4.35 ng/500 U</td>
<td>0.6 ng/100 U</td>
<td>50 ng/5 000 U</td>
</tr>
<tr>
<td>Remaining contents</td>
<td>500 µg albumin</td>
<td>125 µg albumin</td>
<td>1000 µg albumin</td>
<td>0.05% (500µg/ml) albumin</td>
</tr>
<tr>
<td></td>
<td>900 µg sodium chloride</td>
<td>2.5mg lactose</td>
<td>5 mg sucrose</td>
<td>0.1 M sodium chloride</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.01 M sodium succinate&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Form</td>
<td>Vacuum dried powder&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Lyophilised powder&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Lyophilised powder&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Solution&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH</td>
<td>7.3</td>
<td>6.0-7.0</td>
<td>–</td>
<td>5.6</td>
</tr>
<tr>
<td>Storage/Shelf life/Stability</td>
<td>2-8°C or ≤ -5°C</td>
<td>2-8°C</td>
<td>≤ 25°C</td>
<td>2-8°C&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>3 years</td>
<td>2 years</td>
<td>4 years</td>
<td>3 years</td>
</tr>
<tr>
<td></td>
<td>24 h at 2-8°C</td>
<td>8 h at 2-8°C</td>
<td>24 h at 2-8°C</td>
<td>Instant use</td>
</tr>
<tr>
<td>Protein to cleave</td>
<td>SNAP-25</td>
<td>SNAP-25</td>
<td>SNAP-25</td>
<td>VAMP</td>
</tr>
</tbody>
</table>

<sup>a</sup>The manufacturer has not published this information (21).
<sup>b</sup>The product is also available in vials containing 50 U and 200 U.
<sup>c</sup>The product is also available in vials containing 300 U.
<sup>d</sup>The product is also available in vials containing 50 U.
<sup>e</sup>The product is also available in vials containing 2 500 U and 10 000 U.
<sup>f</sup>The product also contains sodium caprylat, sodium acetyltryptoanate, hydrochloric acid and water for injection; the amounts are not listed in the summary of product characteristics.
<sup>g</sup>Physiological sodium chloride (0.9%) is used to dissolve the powder before injection.
<sup>h</sup>The solution is ready to use but can be diluted with physiological sodium chloride (0.9%).
<sup>i</sup>The product can be stored in ≤ 25°C for 3 months but must thereafter be discarded.

The biological activity is expressed in mouse units (U) for all products. By definition, 1 unit is the quantity of BTX that kills 50% of mice by intraperitoneal injection in a mouse lethality assay (LD<sub>50</sub>) (23). However, one unit of each product does not always give the same therapeutic effect when treating humans (18, 23-31). Several studies, performed on α-motorneurons with innervated muscles in humans, demonstrate dissimilar effect between the products. Dose conversion ratios used in the studies referred to above are shown in Table 2.
Table 2. *Dose conversion ratios between different BTX products.*

<table>
<thead>
<tr>
<th>Dose conversion ratio</th>
<th>Botox</th>
<th>Dysport</th>
<th>Xeomin</th>
<th>NeuroBloc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-6</td>
<td>1</td>
<td>50-100</td>
<td></td>
</tr>
</tbody>
</table>

Randomized, controlled trials in cervical dystonia, blepharospasm and hemifacial spasm have been performed comparing Botox and Dysport in dose conversion ratios of 1:3 and/or 1:4 (Botox:Dysport) (25-28). Both dose conversion ratios were found to be too high in one study (25) whereas 1:3 was found suitable to achieve equivalent treatment effect in one trial (26) and 1:4 in two studies (27, 28). Dose conversion ratio 1:4-5 (32), 1:5.3-6 (29) and 1:3 (33, 34) have been suggested in non-randomized, open-label studies when treating patients with cervical dystonia, blepharospasm, hemifacial spasm or spasmodic dysphonia.

Of the studies referred to in Table 2, all except one used solutions that had been diluted to contain dissimilar BTX concentrations. However, Wohlfarth et al. performed both in vitro and in vivo tests using solutions of Botox and Dysport containing the same BTX concentration (24). They also added albumin to the Dysport solution to attain the same amount as in the Botox solution. Under these conditions no difference in effect could be seen, leading to a dose conversion ratio of 1:1 between the products. The same in vitro model was used to investigate the time to muscle paralysis depending on absent or present additional albumin in different solutions of Dysport (100 U/ml and lower) (35). The time to paralysis was prolonged for the solutions without additional albumin and the protection provided by albumin was more pronounced in the lower concentration range. These findings advocate that BTX concentration and albumin content in the injected solution may influence the effect. Albumin is an important component because it prevents adsorption of BTX in the vial and syringe.

The mouse lethality assays are not performed in the same way for the products. The pharmaceutical companies Ipsen and Eisai use a stabilizing gelatine-phosphate buffer when testing Dysport and NeuroBloc in their assays. This is due to the very low concentrations used in the LD50-test, consequently inactivating some of the present BTX when saline is used as a diluent (22, 36). For the same purpose HSA (Human Serum Albumin) have been used for BTX protection when testing Xeomin (37). When saline is used as a diluent, which is the case in the Botox assay, the determination of the potency in the product may result in an underestimation of the units per given amount. This will subsequently lead to an effect that is comparatively higher in clinical practice.

Furthermore, for these biological products the actual amount of BTX, as measured by the LD50-test, is allowed to differ -20% and +25% from the labelled amount in the vial, according to the European pharmacopoeia.
Thereby a vial labelled 100 U may in fact contain an amount somewhere in the range 80-125 U and a vial labelled 500 U may contain 400-625 U. The possible variability between different batches is consequently not insignificant.

Differences in product formulation, performance of the mouse lethality assays and dilution level (concentration) may be factors that cause the divergence in effect between the products. Moreover it is possible that different BTX types, i.e. BTX A versus BTX B, do not affect dissimilar neuron types in the same way. In hyperhidrosis treatment, the BTX affects autonomic sudomotorneurons with innervated sweat glands, while in the treatment of muscular diseases the BTX affects α-motorneurons with innervated muscles.

**Mechanisms of sweating and Hyperhidrosis**

Eccrine sweat glands can be found almost over the entire body surface (38). The function in the hairy skin is to maintain the core temperature and in the glabrous skin in the palms and soles to provide a better grip for physical activity such as “fight or flight”. The eccrine glands are innervated by sympathetic neurons. Acetylcholine is the main transmitter, which provokes sweating after binding to the muscarinic receptor within the gland (38).

Primary focal hyperhidrosis is a condition with excessive sweating in hands, feet, axillae and inguins but also on thermoregulated parts of the body such as head and trunk. Primary hyperhidrosis is a chronic genetic disease with an increased cutaneous sympathetic outflow in response to both mental and thermal stimuli (39, 40). The symptoms may have negative impact on professional life and cause considerably reduced life quality in general (41). However, local intradermal injections of BTX have been shown to improve quality of life for patients suffering from hyperhidrosis (41).

**Cervical dystonia**

Cervical dystonia manifests itself as involuntary muscle contractions that interfere with the movement of the head (42). This may lead to abnormal head and neck postures such as head turning (torticollis), leaning (laterocollis), pulling forward (anterocollis), pulling backward (retrocollis) or a combination of these postures. Neck pain is a common symptom in cervical dystonia. It is thought that genetic factors, trauma, impaired sensory system, and impaired basal ganglia function may play a role in the development of the disease (42). BTX has been used in the treatment of cervical dystonia for several years and is still the therapy of choice for these patients.
Aims

General aim
To find the most appropriate dilution level (concentration) for the commercially available BTX products by measuring sweat reduction in healthy volunteers.

To compare Botox and Dysport in the treatment of cervical dystonia when the products are diluted to contain the same BTX concentration (100 U/ml), without addition of albumin.

Specific aims
- Investigate the sweat reducing effect after intradermal injections of BTX and compare different products and concentrations. This research work consists of two separate studies; a pilot study to identify adequate concentration intervals for the different products followed by a larger study including the most suitable found concentrations. In the second study also the longitudinal changes in effect are investigated.
- Retrospectively investigate the treatment doses, clinical effect and the appearance of adverse events before and after switching BTX product from Botox to Dysport in the treatment of cervical dystonia.
- Prospectively compare Botox (100 U/ml) and Dysport (100 U/ml) in the treatment of cervical dystonia using two different dose conversion ratios.
Material and Methods

Paper I

Nine fully informed and consenting physicians at the department of Neurology were included in the study. All subjects were male and the mean age was 50 years (range 36-65).

Injection procedure

The participants received intradermal injections of Botox, Dysport and NeuroBloc in three different concentrations each. Botox and Dysport were diluted to the concentrations 100 U/ml, 50 U/ml and 25 U/ml and NeuroBloc to 500 U/ml, 250 U/ml and 100 U/ml. Physiological saline was used as diluent. A total of ten 0.1-ml injections were given to every participant, including one control injection of physiological saline without BTX.

The injections were given in the back in three vertical rows. The first row was placed 19 cm (10 cm for 2 persons) in the left lateral direction from the spinal column and the second row 9 cm (5 cm for 2 persons) in the left lateral direction. The third row was placed 9 cm (5 cm for 2 persons) in the right lateral direction from the spinal column. Five participants received injections of Botox in the first row and 4 participants in the second row. Dysport was injected in the first or second row where the participant did not receive Botox. This was in order to exclude differences in effect depending on the position in the back. All participants were given injections of NeuroBloc in the third row. The top injection in each row was given at the same level as the seventh cervical vertebra and the injections beneath were given at 5- to 7-cm intervals.

Iodine-starch test

An iodine-starch test was performed 21 days after the injections (28 days for one person) to identify the area of anhidrotic skin. Iodine alcohol solution was applied on the back of each participant who subsequently entered and stayed in a sauna until a small amount of sweat was visible on the skin. A white sheet of paper (45 g/m²) was thereafter pressed against the back and stained black in contact with wet skin. Anhidrotic areas appeared as white circles on the paper.
Area measuring
Each anhidrotic area was placed under a microscope connected with a camera and a computer. The anhidrotic areas were photographed and thereafter measured in a computer program (Olympus DP-soft, version 3.2).

Anhidrotic mean area and anhidrotic mean area per unit
The anhidrotic mean area was calculated for each concentration, using the participants’ individual result.
Since all injections had a volume of 0.1 ml, the dose differed between the concentrations used. The anhidrotic mean area per unit of each concentration was thereby determined in order to compare the effect of the different injections.

Statistical analyses
A non-parametric test (Mann-Whitney U test) was used for statistical analyses. A p-value < 0.05 was regarded as statistically significant. The analyses were performed with StatView® (SAS institute inc., Cary, NC, USA).

Paper II
This exploratory study was conceived on the basis of Paper I. It was approved by the local ethics committee in Uppsala and the Swedish Medical Products Agency (EudraCT number: 2009-013684-19).
The randomized, double-blind study included 20 consenting subjects of whom 13 were female. The mean age was 36 years (range 19-66 years). None of the subjects had previously received BTX. Ongoing skin disease or local pharmaceutical therapies involving the upper part of the back were criteria for exclusion.

Randomization and injection procedure
At the first study visit all subjects received injections of Botox, Dysport, Xeomin and NeuroBloc diluted with physiological, unpreserved saline. Botox and Xeomin were diluted to the concentrations 100 U/ml, 50 U/ml, 25 U/ml and 10 U/ml, Dysport was diluted to the concentrations 500 U/ml, 200 U/ml, 100 U/ml and 50 U/ml, and NeuroBloc was diluted to the concentrations 500 U/ml, 250 U/ml, 100 U/ml and 50 U/ml. A total of sixteen 0.1-ml injections were given to every subject.
A research nurse diluted the BTX products and prepared sixteen identical syringes, containing the same volume, for each subject. Every syringe was
labelled with an injection point; P1-P16 (see Figure 3), and a subject number. The concentration and product used at the different injection points varied between the subjects in accordance with four different randomization sequences, thus making the study double-blind.

The injections were administered on the back in four vertical rows; P1-P4, P5-P8, P9-P12 and P13-P16, see Figure 3. All four concentrations of each product were injected within the same vertical row, however, the products were allocated to different vertical rows in accordance with the randomization sequence. Furthermore, injections with higher doses of BTX, where large anhidrotic areas were expected, were placed far away from each other to get a distinct border for all injection points. The first vertical row was placed 14 cm in the left lateral direction from the spinal column, the second vertical row 7 cm in the left lateral direction from the spinal column, the third vertical row 7 cm in the right direction from the spinal column and the fourth vertical row 14 cm in the right direction of the spinal column. The top injection in each vertical row was given at the level 5 cm below the seventh cervical vertebra and the injections beneath were given at 6- to 7-cm intervals.

Iodine-starch test
The areas of anhidrotic and hypohidrotic skin were identified with an iodine-starch test 4, 8 and 12 weeks after the injections. Iodine alcohol solution was applied on the back of each participant who subsequently entered and stayed in a sauna until a small amount of sweat was visible on the skin. A white sheet of paper (45 g/m²) was thereafter pressed against the back and stained black in contact with wet skin. Anhidrotic areas appeared as white circles on the paper and hypohidrotic areas, where only a small amount of sweat was present, could be seen as dots at the boarders between the white circles and the black area.

Area measuring
All imprints were scanned and thereafter used in the computer program Adobe Photoshop CS4 where anhidrotic and hypohidrotic areas were measured for each injection point. The data referred to as hypohidrotic area in the Results section, are in fact the anhidrotic area and the hypohidrotic area in total, as measured in the computer program.

Anhidrotic and hypohidrotic mean areas per unit
Since all injections in the study had a volume of 0.1 ml, the dose differed between the concentrations used. The anhidrotic and the hypohidrotic mean
areas per unit, respectively, were therefore calculated for each concentration in order to compare the effect of the different concentrations.

Figure 3. Positions where the 16 injections were given. P1 represents injection point one, P2 represents injection point two and so on.
Primary objective and endpoint

The primary objective of the trial was to study the anhidrotic effect at four weeks after intradermal injections of Botox, Dysport, Xeomin and NeuroBloc diluted to four different concentrations each.

The primary endpoint of the study was the anhidrotic area per unit (cm²/U) for each treatment (i.e. the 16 different product-concentration combinations) at four weeks post treatment.

Secondary objectives

Secondary objectives were to investigate the anhidrotic area per unit at 8 and 12 weeks after intradermal injections of the different products and concentrations and the hypohidrotic area per unit at week 4, 8 and 12. A further objective was to study the longitudinal changes in effect.

Statistical analyses

The sample size estimation in this exploratory study was not based on statistical criteria, but on Paper I and another similar study (43).

The optimal concentration of the four products was evaluated using descriptive statistics and graphs. Optimal concentration was defined as the concentration generating the largest mean anhidrotic area per unit for each product. In addition, treatment comparisons were made by differences of least squares means from a mixed model ANOVA with treatment and position on the back (lateral/medial) as fixed effects and subject as a random effect.

Due to the exploratory nature of this study, no adjustments for multiple comparisons were undertaken. Confidence intervals and p-values were used for exploratory purposes. All statistical analyses were performed with SAS® version 9.3.

Paper III

Study design

A retrospective study using casebook notes from patients with the diagnosis spastic torticollis (cervical dystonia) was undertaken. The administration system INFOMEDIX was used to identify the patients initially treated with Botox, who switched to Dysport around year 2000. To be included in the study the patient had to have received at least four treatments with Botox followed by at least four treatments with Dysport. A patient was excluded if,
according to the casebook notes, not responding to Botox or Dysport. Due to the crossover design all patients acted as their own control. Approval from the ethics committee was not required for the study.

In the initial treatment setting Dysport was given in a dose three times higher than the earlier received Botox dose (dose conversion ratio 1:3, Botox:Dysport). However, this setting caused dysphagia in several of the patients. For this reason it was decided that dose conversion ratio 1:2 (Botox:Dysport) was to be used when changing product for the remaining patients at the clinic. If necessary, an insufficient treatment dose could easily be increased at the patient’s following treatment session. The number of muscles per patient and the amount of each BTX product injected per muscle were chosen at each visit based on clinical judgment and electromyography guidance (44). The clinical appearance of the dystonia did not change during the study; hence the choice of muscles and the dose per muscle were comparable between the treatments.

Approximately one year after the product switch the retrospective analysis was performed in order to establish which doses of Botox and Dysport had been used, respectively, during the change, and whether the doses of Dysport were increased or lowered at subsequent treatments. The subjective clinical effect and the appearance of adverse events were also studied. This will be referred to as the first analysis set.

Six and a half years after the product switch a follow-up was performed to find out whether the doses of Dysport had changed further. The appearance of adverse events was investigated also at this point. This will be referred to as the second analysis set.

Information was drawn from each casebook regarding the administered doses at the last four Botox injections (B1-B4) before the switch to Dysport, the doses used at the first four Dysport injections (D1-D4), and the latest four Dysport doses (D5-D8). Reported adverse events after each of these treatments were captured as well as the patients’ comments concerning the treatment effect (better, worse or the same) after the switch (D1-D4). At the time for the product switch Botox was used in the concentration 100 U/ml and Dysport in the concentration 200 U/ml. Dysport has since 2002 been used in the concentration 100 U/ml.

First analysis set

For each individual the median doses of B1-B4, D1-D4 and D2-D4 were calculated and those values were thereafter used to assess the ratio for B1-B4:D1-D4 and B1-B4:D2-D4, respectively. The median dose ratio was then determined on a group level. The first Dysport dose was excluded to get the median value after the initial dose adjustment. All four Botox values were used since all patients except one had received more than four Botox injections.
The dose ratio for B4:D1 was calculated for each individual and thereafter the median dose ratio was assessed for the entire group.

Second analysis set

Only previously included patients, still treated at the clinic at the time for the follow-up took part in the second analysis set. For each individual the median doses of B1-B4, D2-D4 and D5-D8 were calculated and those values were thereafter used to assess the ratio for B1-B4:D2-D4:D5-D8. The median dose ratio was then determined on a group level.

Statistics

Descriptive statistics have been used throughout the study. Calculations were performed in Microsoft Excel.

Paper IV

Trial design

This double-blind, randomized, cross-over trial was approved by the local ethics committee in Uppsala and the Swedish Medical Products Agency (EudraCT number: 2008-005819-18), and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Informed consent was collected from all subjects.

Inclusion and exclusion criteria

Patients included were 18 years of age or older, diagnosed with cervical dystonia and treated with Dysport regularly for at least one year prior to the study with a stabilized treatment response.

Criteria for exclusion were contraindications to any of the investigational products, pregnancy or lactation, multifocal or generalized dystonia, patients with deep brain stimulation treatment, patients with ongoing pronounced variability in daily benzodiazepine dose (≥ 2 mg clonazepam or corresponding doses of other benzodiazepines), patients suffering from other conditions that, in the opinion of the investigators, might interfere with the study objectives or patients that, in the opinion of the investigators, were unable to comply with study requirements.

Treatments

An overview of the different treatments is shown in Figure 4, next page.
Figure 4. Study flow chart.
* All evaluations of disease severity and treatment effect include TWSTRS (Toronto Western Spasmodic Torticollis Rating Scale), Tsui score, MEI (Movement Energy Index), SF-36 (Short Form-36) and FDQ (Functional Disability Questionnaire).
At the first visit all patients were treated with Dysport and this is referred to as “treatment 1”. The Dysport dose at treatment 1 was based on the individual applied dose at the latest treatment sessions. After treatment 1 the patients were randomized to receive continuing treatment (treatment 2-4) according to one of six study arms. All study arms consisted of the same three treatments but the order in which the different treatments were given varied to keep the involved subjects blinded. The different treatments were as follows: Botox using a dose corresponding to approximately 33% of the given individual Dysport dose at treatment 1 (dose conversion ratio 1:3), Botox using a dose corresponding to approximately 59% of the given individual Dysport dose at treatment 1 (dose conversion ratio 1:1.7), and Dysport using the same individual dose as in treatment 1 (control treatment).

The doses at treatment 2-4 were fixed to provide the dose conversion ratios described above and these doses were calculated on the basis of the dose given at treatment 1. Within this study it was not possible to use other than the fixed doses. The same muscles that were treated during treatment 1 were treated during treatment 2-4, if not judged as severely atrophic, which was a criterion for exclusion. Electromyographic guidance was used for all injections. Adverse events were captured at all visits with general questions and specific inquiries about neck weakness, dry mouth and dysphagia.

Blinding procedure and study medication preparation

The patients and the personnel involved in the study were blinded throughout the trial period. Patients were randomized to six different treatment sequences with Botox (dose conversion ratio 1:3), Botox (dose conversion ratio 1:1.7) and Dysport using the treating physician as a stratification factor. Randomization was performed by a statistician in blocks of 6.

Three physicians (JB, AJ, DN) treated about 1/3 each of the patients. The physicians prepared BTX prescription cards for each other’s patients to remain blinded. Six nurses prepared BTX solution in syringes according to the prescription card. The syringes were marked with a tag indicating the muscle in which the whole content in one syringe was injected by the blinded physician. The concentration of BTX was 100 U/ml for both products during the entire study. Physiological sodium chloride was used as diluent.

BTX responsiveness

BTX responsiveness was confirmed with two separate injections of Botox (7.5 units x 2) in one side of the forehead (frontalis test) at the last study treatment (45). The test was evaluated 4 weeks after injection when the patients were visiting the hospital for evaluation of the study treatment.
Instruments for evaluation

Five different instruments were used to evaluate the effect four weeks after each treatment and the disease severity 12 weeks after each treatment. An overview of the different treatments is shown in Figure 4.

Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) consists of three domains that evaluate clinical severity, disability and pain (46, 47). The maximum score for each domain is 35, 30 and 20, resulting in a total maximum score of 85 representing worst imaginable difficulty due to the disease.

The Tsui scale contains four domains that evaluate amplitude and duration of sustained movements, shoulder elevation and tremor (48). The maximum total score is 25 representing worst imaginable difficulty due to the disease.

Movement Energy Index (MEI) estimates the mechanical power and work involved in the movements of the head calculated from optoelectronic data (49). The patient is seated in front of a cross-shaped target on a white screen. A headband, with an attached laser pointer and a cluster with four markers, is firmly fastened around the patient’s head. While the patient performs a standardized set of movements along the target on the screen, four cameras measure the position of the reflective markers on the headband. The detection of the movements can thereafter be used to calculate the MEI.

Short Form-36 (SF-36) is a standardized form consisting of 36 questions divided into 8 different health domains (50). The maximum score for each domain is 100, which represents best imaginable health. From the 8 domains two different summary measures, physical health and mental health, are calculated.

Functional Disability Questionnaire (FDQ) lists 27 commonly performed daily activities (51). The patients notify which of the activities are affected by the cervical dystonia and score the extent of impact. The maximum score is 108, which represents worst imaginable difficulty in performing daily activities due to the disease. The total score was used in this study.

All these evaluations were performed by a physicist and two registered physiotherapists with substantial experience in evaluating patients with cervical dystonia by using the instruments. The order in which the instruments were used was the same for all patients throughout the study.

Objectives

Primary objective

To evaluate the difference in treatment effect between Botox and Dysport for dose conversion ratio 1:3 using the total score of TWSTRS.
Secondary objectives

To evaluate the difference in treatment effect between Botox and Dysport for dose conversion ratio 1:1.7 using TWSTRS (total plus subscores for torticollis severity, disability and pain), Tsui score, MEI, SF-36 and FDQ.

To evaluate the difference in treatment effect between Botox and Dysport for dose conversion ratio 1:3 using TWSTRS subscores for torticollis severity, disability and pain, Tsui score, MEI, SF-36 and FDQ.

To evaluate the difference in disease severity between Botox and Dysport for dose conversion ratio 1:1.7 and 1:3 using TWSTRS (total plus subscores for torticollis severity, disability and pain), Tsui score, MEI, SF-36 and FDQ.

Endpoints

Primary endpoint

Treatment effect measured by TWSTRS total score four weeks after each treatment.

Secondary endpoints

TWSTRS subscores for torticollis severity, disability and pain, Tsui score, MEI, SF-36 and FDQ four weeks after each treatment.

TWSTRS (total plus subscores for torticollis severity, disability and pain), Tsui score, MEI, SF-36 and FDQ twelve weeks after each treatment.

Statistics

Determination of sample size

The sample size calculation was performed assuming paired t-test for mean difference (SAS 9.1). It was assumed that the intraclass correlation coefficient of the pairwise measurements of TWSTRS total score was 0.5 and that the standard deviation for the mean score of TWSTRS total was 11.0. Based on a previous study, we assumed 4 points on the TWSTRS total scale would reflect a minimal clinically relevant change (52). To detect a difference of 4 points on the TWSTRS total score between Botox and Dysport (dose conversion ratio 1:3) with 80% power and \( \alpha = 0.05 \) a sample size of 62 subjects was required.

The TWSTRS total score is a non-normally distributed response variable but the sample size calculation was based on a parametric test. We needed to
account for not using a parametric test (10%) and for dropouts (10%). The estimated total sample size required for this study was 75 subjects.

**Analysis of primary endpoint**

The primary analysis of the difference in TWSTRS total score between Botox (dose conversion ratio 1:3) and Dysport, four weeks after treatment, consisted of a Wilcoxon-Mann-Whitney rank sum test with Koch’s method (53) to adjust for period effects. The six treatment sequences were arranged in pairs. Within each pair Botox (dose conversion ratio 1:3) and Dysport occurred in the same periods. Each of the three pairs of the sequences formed a two treatment-two period cross-over. The analysis consisted of two stages, first performing a Wilcoxon-Mann-Whitney rank sum test using Koch’s method on each of the three 2 x 2 cross-overs formed by pairing the sequences, and secondly combining the results from each analysis (54). A Hodge-Lehman type estimator and confidence interval of approximately 95% of the treatment effect were calculated (54).

A sensitivity analysis was performed using a mixed model ANOVA with TWSTRS total score as response variable, period and treatment as fixed effects and patient as a random effect. The estimate of the treatment difference (Botox (1:3) – Dysport) with a 95% CI was calculated.

All tests of treatment difference were two-sided and performed at 5% significance level. TWSTRS scores are also presented by treatment. All data were primarily analysed on an intention to treat basis and per protocol analysis was used for confirmation.

**Secondary efficacy analyses**

The secondary endpoints were analysed in the same way as the primary endpoint except that no sensitivity analyses were performed.

**Post-hoc analysis**

A post-hoc analysis concerning the patients’ assessments of the treatment effect from each of the different study treatments was performed before unblinding the physicians. The assessments were captured at each treatment visit, i.e. 12 weeks after previous treatment, noted in the patients’ case books and thereafter classified according to a 5-grade scale where 1 represents very poor or no treatment effect and 5 represents very good treatment effect.
Results

Paper I

The results are based on the imprints from the 9 included subjects. One participant received less than 0.1 ml of Dysport 100 U/ml during the injection; therefore that anhidrotic area was excluded in the results. Furthermore, for another participant one imprint from where Botox 100 U/ml had been injected was indistinct due to a crease on the paper, consequently that anhidrotic area was excluded in the results. However, the rest of the data from these two participants were included in the analyses. Mean values are therefore based on the data from 9 subjects for all products and concentrations except for Dysport 100 U/ml (n=8) and Botox 100 U/ml (n=8).

The anhidrotic mean area for each product and concentration is shown in Table 3.

Table 3. Anhidrotic mean area for each product and concentration.

<table>
<thead>
<tr>
<th>Botox</th>
<th>Dysport</th>
<th>NeuroBloc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (dose)*</td>
<td>Anhidrotic mean area</td>
<td>Concentration (dose)*</td>
</tr>
<tr>
<td>100 U/ml (10 U)</td>
<td>3.5 cm²</td>
<td>100 U/ml (10 U)</td>
</tr>
<tr>
<td>50 U/ml (5 U)</td>
<td>2.6 cm²</td>
<td>50 U/ml (5 U)</td>
</tr>
<tr>
<td>25 U/ml (2.5 U)</td>
<td>1.7 cm²</td>
<td>25 U/ml (2.5 U)</td>
</tr>
</tbody>
</table>

*Injected dose for 0.1 ml solution.

The anhidrotic mean area per unit for each product and concentration is shown in Table 4 and Figure 5. A statistically significant difference manifested itself between Botox 25 U/ml and Botox 100 U/ml (p<0.01), Botox 50 U/ml and Botox 100 U/ml (p=0.02), NeuroBloc 100 U/ml and NeuroBloc 500 U/ml (p=0.04) and between NeuroBloc 250 U/ml and NeuroBloc 500 U/ml (p<0.01).
Table 4. Anhidrotic mean area per unit for each product and concentration.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Botox Anhidrotic mean area per unit</th>
<th>Dysport Anhidrotic mean area per unit</th>
<th>NeuroBloc Anhidrotic mean area per unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 U/ml</td>
<td>0.35 cm$^2$/U</td>
<td>0.30 cm$^2$/U</td>
<td>0.14 cm$^2$/U</td>
</tr>
<tr>
<td>50 U/ml</td>
<td>0.52 cm$^2$/U</td>
<td>0.30 cm$^2$/U</td>
<td>0.25 cm$^2$/U</td>
</tr>
<tr>
<td>25 U/ml</td>
<td>0.69 cm$^2$/U</td>
<td>0.30 cm$^2$/U</td>
<td>0.31 cm$^2$/U</td>
</tr>
</tbody>
</table>

Figure 5. Size of the anhidrotic area per unit of skin after injections of various doses of Botox, Dysport and NeuroBloc. Mean values and standard deviations areas are shown for each concentration.

To investigate the difference in sweat reducing effect between the three products the anhidrotic mean areas after injections with 10 units (100 U/ml) of each product were compared. The calculated conversion ratios were 1:1.2 (Botox:Dysport) and 1:1.1 (Botox:NeuroBloc).

Paper II
Eighteen of the 20 included subjects completed the study. Two of the participants were not able to attend the follow-up visit at week 12; however the
data from week 4 and 8 are included in the analyses. Due to indistinct sections of some imprints, it was not possible to measure 217 of 928 injection points. The results are thereby based on 711 injection points. The exclusion was made before un-blinding.

The mean anhidrotic area per unit four weeks after injection of the four different products and concentrations is presented in Figure 6. The optimal concentration for both Botox and Xeomin was 25 U/ml. The differences between Botox 25 U/ml and the other three Botox concentrations were statistically significant (Botox 10 U/ml p=0.0015, Botox 50 U/ml p=0.0038 and Botox 100 U/ml p=0.0003); this was also seen for Xeomin 25 U/ml when comparing to the other three Xeomin concentrations (Xeomin 10 U/ml p=0.0048, Xeomin 50 U/ml p=0.0387 and Xeomin 100 U/ml p=0.0039). The largest mean anhidrotic area per unit for Dysport appeared where the concentration 100 U/ml had been injected, however, the difference between Dysport 100 U/ml and Dysport 50 U/ml was not statistically significant (p=0.2200), nor was the difference between Dysport 100 U/ml and 200 U/ml (p=0.9711) (Figure 6). The optimal concentration seems to be about 100 U/ml. A statistically significant difference manifested itself between Dysport 100 U/ml and Dysport 50 U/ml (p=0.0135). The optimal concentration for NeuroBloc was 50 U/ml. The differences between NeuroBloc 50 U/ml and the other three NeuroBloc concentrations were statistically significant (NeuroBloc 100 U/ml p=0.0105, NeuroBloc 250 U/ml p=0.0002 and NeuroBloc 500 U/ml p<0.0001).

When comparing the mean anhidrotic area per unit obtained after injection of the same concentration and dose for each product the calculated dose conversion ratios were 1:1.6:1.2:1.3 (Botox:Dysport:Xeomin:NeuroBloc) for concentration 100 U/ml (10 U). The difference in mean anhidrotic area per unit between Botox 100 U/ml and Dysport 100 U/ml was statistically significant (p=0.0162).

For concentration 50 U/ml (5 U) the dose conversion ratios were 1:3.3:1.2:1:1 (Botox:Dysport:Xeomin:NeuroBloc). The mean anhidrotic area per unit obtained by Dysport 50 U/ml was significantly smaller compared to all other products (Botox 50 U/ml p<0.0001, Xeomin 50 U/ml p=0.0001 and NeuroBloc 50 U/ml p=0.0002). Between the other three products there were no statistically significant differences (Botox 50 U/ml – Xeomin 50 U/ml p=0.2763, Botox 50 U/ml – NeuroBloc 50 U/ml p=0.4381 and Xeomin 50 U/ml – NeuroBloc 50 U/ml p=0.8054).
Figure 6. Upper panel: the mean anhidrotic area per unit four weeks after injection of Botox, Dysport, Xeomin and NeuroBloc in four different concentrations. Clear peaks can be seen for Botox and Xeomin, demonstrating the optimal concentration 25 U/ml for both products. The optimal concentration for Dysport is approximately 100 U/ml and for NeuroBloc 50 U/ml. Lower panel: enlargement to make explicit the mean anhidrotic area per unit for Dysport and NeuroBloc at week four.
As comparing the optimal concentration for each product, i.e. Botox 25 U/ml, Dysport 100 U/ml, Xeomin 25 U/ml and NeuroBloc 50 U/ml the dose conversion ratios were 1:4.8:1.3:2.2 (Botox:Dysport:Xeomin:NeuroBloc). The mean anhidrotic area per unit obtained by Dysport 100 U/ml was significantly smaller compared to all other products (Botox 25 U/ml p<0.0001, Xeomin 25 U/ml p=0.0019 and NeuroBloc 50 U/ml p=0.0016) and NeuroBloc 50 U/ml was significantly smaller than Botox 25 U/ml (p=0.0018).

The difference between Botox 25 U/ml and Xeomin 25 U/ml was not statistically significant (p=0.2490), nor was the difference between Xeomin 25 U/ml and NeuroBloc 50 U/ml (p=0.0589).

A secondary objective was to investigate the hypohidrotic area per unit of the four different products and concentrations. The results, four weeks after injection, are presented in Figure 7.

The relationship between the different concentrations within each product showed approximately the same pattern as for anhidrotic area per unit four weeks after injection. Compared to the graph exhibiting the anhidrotic area per unit it can be noticed that Dysport 50 U/ml is closer to the concentrations 100 U/ml and 200 U/ml. All these three hypohidrotic mean areas per unit were significantly larger than the hypohidrotic mean area per unit appearing after injection of Dysport 500 U/ml (Dysport 50 U/ml p=0.0142, Dysport 100 U/ml p=0.0011 and Dysport 200 U/ml p=0.0007). There were, on the other hand, no significant differences between Dysport 50 U/ml, 100 U/ml and 200 U/ml (Dysport 50 U/ml – Dysport 100 U/ml p=0.9508, Dysport 50 U/ml – Dysport 200 U/ml p=0.6868 and Dysport 100 U/ml – Dysport 200 U/ml p=0.6362).
Figure 7. Upper panel: the mean hypohidrotic area per unit four weeks after injection of Botox, Dysport, Xeomin and NeuroBloc in four different concentrations. Clear peaks can be seen for Botox and Xeomin, demonstrating the optimal concentration 25 U/ml for both products. There is no apparent difference between Dysport 50 U/ml, 100 U/ml and 200 U/ml. The mean hypohidrotic area per unit emerging where Dysport 500 U/ml have been injected is, however, smaller compared to the other three Dysport concentrations. Optimal concentration for NeuroBloc is 50 U/ml. Lower panel: enlargement to make explicit the mean hypohidrotic area per unit for Dysport and NeuroBloc at week four.
A further objective was to study the longitudinal changes in effect.

At week 8 it was clearly seen that the optimal concentration still was 25 U/ml for Botox and Xeomin and 50 U/ml for NeuroBloc (Figure 8). The mean anhidrotic area per unit for Dysport 50 U/ml, 100 U/ml and 200 U/ml was approximately the same.

![Mean anhidrotic area per unit (cm²/U), week 8](image)

Figure 8. The mean anhidrotic area per unit eight weeks after injection of Botox, Dysport, Xeomin and NeuroBloc in four different concentrations. Clear peaks can be seen for Botox and Xeomin, demonstrating the optimal concentration 25 U/ml for both products. There is no apparent difference between Dysport 50 U/ml, 100 U/ml and 200 U/ml. The mean anhidrotic area per unit emerging where Dysport 500 U/ml have been injected is, however, smaller compared to the other three Dysport concentrations. Optimal concentration for NeuroBloc is 50 U/ml.

The graph at week 12 is similar to the graph at week 4 and 8, showing that the optimal concentration was 25 U/ml for Botox and Xeomin and 100 U/ml for Dysport (Figure 9). However, the mean anhidrotic area per unit obtained after injection of NeuroBloc was at this measuring time point nearly the same for all four concentrations.

The results considering the mean hypohidrotic area per unit at week 8 and week 12 were in congruence with the results of the mean anhidrotic area per unit for the corresponding weeks, with the exception that Botox in the concentrations 10 U/ml and 25 U/ml were approximately the same.
Figure 9. The mean anhidrotic area per unit twelve weeks after injection of Botox, Dysport, Xeomin and NeuroBloc in four different concentrations. Clear peaks can be seen for Botox and Xeomin, demonstrating the optimal concentration 25 U/ml for both products. One, presumably erroneous, outlying observation for Xeomin 10 U/ml has been omitted. The deletion of this observation altered the mean anhidrotic area per unit for Xeomin 10 U/ml from 0.49 to 0.24 cm²/U. The optimal concentration for Dysport is 100 U/ml. The mean anhidrotic area per unit emerging where NeuroBloc have been injected is nearly constant for all four concentrations.

Six patients reported adverse events related or possibly related to the study interventions. One participant reported local burning sensations when receiving the injections of BTX. Five of the participants experienced dryness of the skin on the back a couple of days after the iodine-starch test, which could have been due to the alcohol containing iodine solution.

Paper III

129 patients with the diagnosis spastic torticollis (cervical dystonia) were identified, see Figure 10. Fifty-four of these patients were excluded; 20 patients did not respond to Botox anymore, 19 patients had never received Dysport, 14 patients had received less than four injections of Botox or Dysport, and 1 patient was mistreated and hospitalised after the product switch. Thereby 75 patients were included in the study. About two-thirds of the patients were women. The age varied between 24-85 years.
At the time for the follow-up, 6½ years after the product switch, 53 of the same patients were included. Twenty-two patients could not be included in this part of the study (7 patients had moved away and continued the treatment at another hospital, 2 patients were deceased, 2 patients had started another kind of treatment, 7 patients did not use Dysport anymore because of absent clinical effect, 1 patient did, for unknown reason, not get treatment with BTX any longer, and for 3 patients the casebooks were not available at this time).

**First analysis set (n=75)**

The doses of Botox (B1-B4) and Dysport (D1-D4) used in relation to the product switch are presented in Table 5.
Table 5. Doses (units) of Botox and Dysport used in relation to the product switch.

<table>
<thead>
<tr>
<th></th>
<th>Median dose</th>
<th>Min</th>
<th>q1</th>
<th>q3</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First analysis set (n=75)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1-B4*</td>
<td>97.5</td>
<td>40</td>
<td>82.5</td>
<td>120</td>
<td>200</td>
</tr>
<tr>
<td>D1-D4*</td>
<td>200</td>
<td>80</td>
<td>180</td>
<td>275</td>
<td>420</td>
</tr>
<tr>
<td>D2-D4*</td>
<td>200</td>
<td>80</td>
<td>160</td>
<td>280</td>
<td>420</td>
</tr>
<tr>
<td>B4</td>
<td>100</td>
<td>30</td>
<td>82.5</td>
<td>120</td>
<td>240</td>
</tr>
<tr>
<td>D1</td>
<td>220</td>
<td>80</td>
<td>180</td>
<td>290</td>
<td>600</td>
</tr>
<tr>
<td><strong>Second analysis set (n=53)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1-B4*</td>
<td>95</td>
<td>60</td>
<td>82.5</td>
<td>112.5</td>
<td>170</td>
</tr>
<tr>
<td>D2-D4*</td>
<td>200</td>
<td>100</td>
<td>160</td>
<td>240</td>
<td>400</td>
</tr>
<tr>
<td>D5-D8*</td>
<td>175</td>
<td>75</td>
<td>135</td>
<td>210</td>
<td>500</td>
</tr>
</tbody>
</table>

*Based on each individual’s median dose.
B1-B4 indicate Botox doses 1-4; D1-D4, Dysport doses 1-4, and so on.

The median dose ratio for B1-B4:D1-D4 was 1:2.2, for B1-B4:D2-D4 1:2.1 and for B4:D1 1:2.3.

Adverse events of B1-B4 were found in the casebook notes for four patients (5.3%); two of them were dysphagia and the other two were pain and dizziness.

Eighteen patients (24%) reported in total 20 adverse events after the switch to Dysport (D1-D4), 8 of them reported dysphagia. Other reported adverse events were; neck weakness (4), pain (4), dizziness (2), tremor (1) and nausea (1).

Neck weakness and dysphagia are adverse events attributable to dose-related muscular and salivatory glandular effects of BTX. The median value of the received Dysport doses (D1-D4) for the patients who suffered from dysphagia or neck weakness (n=11) was 260 U (q1 190 U, q3 360 U, range 115-420 U). At the transition from Botox to Dysport the median dose ratio for B1-B4:D2-D4 was 1:2.4 and for B4:D1 1:2.5.

For the patients who did not experience dysphagia or neck weakness the median dose of Dysport (D1-D4) was lower, 200 U (Q1 177.5 U, Q3 262.5 U, range 80-400 U). The median dose ratio for B1-B4:D2-D4 was 1:2.1 and for B4:D1 1:2.2.

Twelve patients (16%) thought they had a more effective treatment after the switch to Dysport, 4 patients (5%) found the effect worse than earlier and the rest, 59 patients (79%), felt no difference at all in effect.

**Second analysis set (n=53)**

The doses of Botox (B1-B4) and Dysport (D2-D4 and D5-D8) used at the follow-up are presented in Table 5 and Figure 11. The median dose ratio for B1-B4:D2-D4:D5-D8 was 1:2.1:1.7.
During the treatment with Dysport D5-D8 six patients (11.3%) reported in total seven adverse events; four patients reported pain, two patients reported dysphagia and one patient reported heavy breathing.

Figure 11. Doses of Botox (B1-B4) and Dysport (D1-D4) used in relation to the product switch and at the follow-up (D5-D8) for the 53 patients included in the second analysis set. Outliers are denoted by circles and extremes by asterisks.

Paper IV
At the time for subject inclusion 123 patients with cervical dystonia were treated at the department of Neurology, Uppsala University Hospital. Of the patients with diagnosed cervical dystonia, 73 met the inclusion and exclusion criteria but only 48 patients consented to participate in the study; therefore the planned sample size of 75 patients was reduced. Of the 48 included patients 46 patients were randomized at visit 2 (one of the included patients withdrew informed consent and one patient, with a reported stable response to treatment but still clinically suspected resistance to BTX, exhibited a negative frontalis test 4 weeks after visit 1). Of the 46 randomized patients, 43 completed the study (one patient withdrew informed consent at visit 3 due to
worsening symptoms and two patients were excluded at visit 4 due to pronounced muscle atrophy).

The mean age of the patients was 62 years (SD 11 years, range 33-84), mean duration of disease 17 years (SD 10 years, range 2-48) and the mean time since first-ever BTX injection for cervical dystonia 12 years (SD 5 years, range 2-21). The median pre-treatment TWSTRS total score was 43.75 points (range 16.00-62.75). The mean dose of Dysport given at treatment 1 was 169 units (SD 63 units, range 50-400).

Descriptive statistics are presented in Table 6 and Figure 12.

Table 6. Descriptive statistics.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time point</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>TWSTRS total</td>
<td>Pre-treatment</td>
<td>41.62</td>
<td>13.51</td>
<td>43.75</td>
<td>16.00-62.75</td>
</tr>
<tr>
<td>Botox (1:3)</td>
<td>Week 4</td>
<td>35.75</td>
<td>12.76</td>
<td>34.25</td>
<td>11.00-65.00</td>
</tr>
<tr>
<td>Botox (1:1.7)</td>
<td>Week 4</td>
<td>34.60</td>
<td>13.88</td>
<td>34.25</td>
<td>0.00-61.75</td>
</tr>
<tr>
<td>Botox (1:1.7)</td>
<td>Week 12</td>
<td>39.81</td>
<td>14.49</td>
<td>38.38</td>
<td>14.00-71.75</td>
</tr>
<tr>
<td>Dysport</td>
<td>Week 4</td>
<td>33.86</td>
<td>14.04</td>
<td>34.50</td>
<td>6.00-63.75</td>
</tr>
<tr>
<td>Dysport</td>
<td>Week 12</td>
<td>36.88</td>
<td>14.61</td>
<td>38.88</td>
<td>9.00-66.25</td>
</tr>
<tr>
<td>Tsui</td>
<td>Pre-treatment</td>
<td>10.3</td>
<td>4.8</td>
<td>10.0</td>
<td>2-21</td>
</tr>
<tr>
<td>Botox (1:3)</td>
<td>Week 4</td>
<td>8.1</td>
<td>3.9</td>
<td>8.0</td>
<td>2-18</td>
</tr>
<tr>
<td>Botox (1:1.7)</td>
<td>Week 4</td>
<td>7.6</td>
<td>3.8</td>
<td>8.0</td>
<td>0-16</td>
</tr>
<tr>
<td>Botox (1:1.7)</td>
<td>Week 12</td>
<td>9.0</td>
<td>4.2</td>
<td>10.0</td>
<td>1-20</td>
</tr>
<tr>
<td>Dysport</td>
<td>Week 4</td>
<td>7.5</td>
<td>4.0</td>
<td>7.0</td>
<td>1-17</td>
</tr>
<tr>
<td>Dysport</td>
<td>Week 12</td>
<td>8.8</td>
<td>4.6</td>
<td>8.0</td>
<td>1-20</td>
</tr>
<tr>
<td>MEI</td>
<td>Pre-treatment</td>
<td>0.809</td>
<td>1.174</td>
<td>0.430</td>
<td>0.154-7.679</td>
</tr>
<tr>
<td>Botox (1:3)</td>
<td>Week 4</td>
<td>0.631</td>
<td>0.903</td>
<td>0.300</td>
<td>0.108-4.388</td>
</tr>
<tr>
<td>Botox (1:1.7)</td>
<td>Week 4</td>
<td>0.543</td>
<td>0.627</td>
<td>0.259</td>
<td>0.112-3.287</td>
</tr>
<tr>
<td>Botox (1:1.7)</td>
<td>Week 12</td>
<td>0.721</td>
<td>1.388</td>
<td>0.242</td>
<td>0.098-8.627</td>
</tr>
<tr>
<td>Dysport</td>
<td>Week 4</td>
<td>0.476</td>
<td>0.499</td>
<td>0.244</td>
<td>0.117-2.381</td>
</tr>
<tr>
<td>Dysport</td>
<td>Week 12</td>
<td>0.582</td>
<td>0.831</td>
<td>0.273</td>
<td>0.122-5.299</td>
</tr>
</tbody>
</table>

TWSTRS (Toronto Western Spasmodic Torticollis Rating Scale), MEI (Movement Energy Index).
Figure 12. Boxplots presenting the total score of TWSTRS (Toronto Western Spasmodic Torticollis Rating Scale) at pre-treatment, week 4 and week 12.

The primary analysis showed that the estimated median TWSTRS total score was 1.96 points higher for Botox (1:3) compared to Dysport at week 4, the difference was not statistically significant (p=0.0799). The results from the different analyses of TWSTRS total are summarised in Table 7.
No statistically significant differences were observed in the data from the other four secondary measurements.

The results from the post-hoc analysis, concerning the patients’ assessment of the treatment effect, are presented in Figure 13.

The overall occurrence of adverse events was similar with the different treatments. However, there were a higher number of events of dry mouth after the Botox (1:3) treatment. Table 8 presents the overall occurrence of adverse events and of dysphagia, dry mouth and neck weakness.

The frontalis test at the last study treatment showed that three patients in the study did not respond to Botox. Another two patients did not accept to do this test and three patients did not do the test due to early termination in the study. The intention to treat analysis and the per protocol analysis (where the non-responding and the early terminating patients were excluded) showed, however, similar results.
Figure 13. The patients’ assessment of treatment effect from each of the different study treatments. The comments were captured at 12 weeks after previous treatment, noted in the patients’ case books and thereafter classified according to a 5-grade scale where 1 represents very poor or no treatment effect and 5 represents very good treatment effect.

Table 8. Adverse events (AE).

<table>
<thead>
<tr>
<th>AE type</th>
<th>Events/n (%)</th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Botox (1:3)</td>
<td>Botox (1:1.7)</td>
<td>Dysport</td>
<td>Dysport pre-randomization</td>
<td></td>
</tr>
<tr>
<td>n=45</td>
<td>n=43</td>
<td>n=44</td>
<td>n=46</td>
<td>n=46</td>
<td></td>
</tr>
<tr>
<td>Any AE</td>
<td>62/29 (64.4)</td>
<td>74/30 (69.8)</td>
<td>65/27 (61.4)</td>
<td>36/24 (52.2)</td>
<td>237/41 (89.1)</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>7/7 (15.6)</td>
<td>9/9 (20.9)</td>
<td>7/7 (15.9)</td>
<td>3/3 (6.5)</td>
<td>26/15 (32.6)</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>12/12 (26.7)</td>
<td>6/6 (14.0)</td>
<td>4/4 (9.1)</td>
<td>5/5 (10.9)</td>
<td>27/18 (39.1)</td>
</tr>
<tr>
<td>Neck weakness</td>
<td>11/11 (24.4)</td>
<td>15/15 (34.9)</td>
<td>12/12 (27.3)</td>
<td>6/6 (13.0)</td>
<td>44/24 (52.2)</td>
</tr>
</tbody>
</table>

n is the number of patients with at least one AE, percent is calculated as number of patients with at least one AE divided by the total number of patients in the treatment group.
Discussion and Conclusions

Paper I and Paper II

Paper I showed that the lower concentrations of Botox (25 U/ml) and NeuroBloc (100 U/ml) in the chosen concentration interval lead to a relatively enhanced anhidrotic effect. There was no difference between the anhidrotic mean areas per unit comparing the 3 different concentrations of Dysport.

Paper II clearly showed that 25 U/ml is the optimal concentration for Botox and Xeomin to reduce sweating. For Dysport the optimal concentration was approximately 100 U/ml. No clear peak effect was seen for Dysport in this study with small differences in potency for the concentrations 50 U/ml, 100 U/ml and 200 U/ml. However, Dysport 100 U/ml was more effective than Dysport 500 U/ml. A larger cohort study would increase the chances to capture the most appropriate concentration to use for Dysport, which seems to be about 100 U/ml. For NeuroBloc the optimal concentration was 50 U/ml, however, considering the shape in the graph (Figure 6), the optimal concentration may be even lower. Future studies with NeuroBloc should include the concentrations 10 U/ml, 25 U/ml, 50 U/ml and 100 U/ml, in the search for a peak effect and an optimal concentration for the BTX B product.

NeuroBloc and the BTX A products had a similar anhidrotic effect, seen in both Paper I and II, but the effect of NeuroBloc seemed to diminish more prominently at the measurement week 12, as shown in Paper II. This is in accordance with previous clinical studies (55, 56).

A serotype effect in which NeuroBloc was autonomic sudomotorneuron specific was observed in both Paper I and II; this has also been seen in other studies (55, 57). Serotype A (Botox, Dysport and Xeomin) and serotype B (NeuroBloc) have dissimilar acceptor bindings on the neurons and affect different enzymes (SNAP-25 and VAMP, respectively) which may explain varying specificity.

When comparing the dose conversion ratios between BTX A and BTX B for anhidrotic effect (ratio 1:1-2) and relaxing effect on muscles (ratio 1:50-100), an opportunity to treat special groups with hyperhidrosis becomes apparent. Dilution enables treatment of general hyperhidrosis with large areas such as the head and trunk, without exceeding the maximum dose. Furthermore, small doses reduce the risk for side-effects, especially from underlying muscle tissue when treating sensitive parts of the body as the central face and palms. The benefits of using low concentrations of BTX can likewise be
seen for the other products, although not in the same extent as for Neur-robloc. Paper I and Paper II, as well as other studies, show that the level of dilution of the products is important for optimal effect (24, 43, 58).

Even though Botox and Dysport are approved for treatment of axillary hyperhidrosis, most dose-response studies are still performed on muscles and α-motorneurons, which differ from autonomic sudomotorneurons. Allergan (Botox) and Ipsen (Dysport) have used predetermined doses for axillary hyperhidrosis, which is sufficient for good results on a group basis. However, when treating a chronic disease such as hyperhidrosis, it is convenient to use an individual and minimal dose with an optimal effect, partly to reduce the risk of side-effects and immunization, partly to reduce the costs.

As far as we know, the medical companies have no assay for studying the effect of BTX on autonomic sudomotorneurons. The method used in Paper I and II is very simple and easy to perform with reproducible results. Kranz et al. have compared the sweat reducing effect of Botox and Dysport with corresponding results (43). They have used a similar method but chose abdominal skin, which provides homogenous sweating conditions. We performed the measuring on the back in favour of the even surface and less hairy skin. Pitfalls with this method are; how to stimulate sweating in a standardized manner, how to compensate for different levels of sweating on the back, and finally, how to exclude small anhidrotic/hypohidrotic areas, which can be artifacts.

We used heat stimulation in a sauna, as sweating from the trunk is thermo-regulated. When the sweating from the back started, an iodine-starch imprint was performed. It was not important to keep external factors such as the temperature in the sauna and the duration of the visit in the sauna constant between subjects since the rate of sweating was individual and the quality of the imprints would be inadequate if the back was too dry or too wet when performing the iodine-starch test. However, on an individual basis the autonomic sudomotorneuron signaling to the sweat glands on the back was homogenous, which allowed us to compare the different measuring points. It is well known that sweating is more prominent in the middle of the back, down the spine, compared to lateral parts of the back. We compensated this heterogeneity by altering position for injection (lateral/medial) for Botox/Dysport between the subjects in Paper I and by randomizing the subjects to different treatment sequences in Paper II; this minimized any potential bias. Additionally in Paper II, the position on the back (lateral/medial) was incorporated in the statistical models, hence adjusting the treatment effects for position effects.

In Paper II approximately 20% of the imprints were excluded because of uncertain margins and artifacts due to too much or too little sweat on the back. To eliminate possible bias the exclusion was done before un-blinding.

In contrast to Paper I, both anhidrotic and hypohidrotic areas were measured on the imprints in Paper II. Anhidrosis was primarily monitored since
the distinct border makes it easier to measure the area and thereby provides a more precise result. However, as a secondary outcome, hypohidrotic area was measured since this may be more relevant in the clinical practice. Hypohidrosis can be a more desirable result than anhidrosis in the treatment of patients with hyperhidrosis since the patients consider it slightly negative if the skin is too dry (14). This study as well as the study performed by Kranz et al., showed a high correspondence between anhidrotic and hypohidrotic areas after injection of BTX (43).

For a clinician it is necessary to have a dose- and concentration regimen for each product, especially if switching between them. Dose conversion ratios between Botox and Dysport have, in the literature, been reported to vary considerably among different studies performed on muscles. The dose conversion ratio has been found to be 1:1–6 (Botox:Dysport).

In the following four studies (26-29) the concentrations, however, have been dissimilar, conceivably causing the different results:

Odergren et al. concluded that the dose conversion ratio was 1:3 (Botox:Dysport) when the concentration for Botox was 100 U/ml and for Dysport 300 U/ml.

Sampaio et al. 1:4 (Botox:Dysport) when using Botox 25 U/ml and Dysport 200 U/ml.

Nüssgens et al. 1:4 (Botox:Dysport) when using Botox 25 U/ml and Dysport 100 U/ml.

Durif et al. 1:5.3-6 (Botox:Dysport) when using Botox 25 U/ml and Dysport 200 U/ml.

On the other hand, when both Botox and Dysport were diluted to the concentration 100 U/ml and the solution contained the same amount of albumin there was no difference in effect, investigated on muscles in healthy volunteers and in vitro (24).

Paper I and Paper II, which are performed on sweating and autonomic sudomotorneurons, give further evidence that it is crucial to consider the concentration of BTX when comparing different products. When diluting all products to the concentration 100 U/ml, the clinical effect was relatively similar.

In contrast, when comparing the mean anhidrotic area per unit obtained after injection of 50 U/ml, in Paper II, the calculated dose conversion ratio was 1:3.3 for Botox:Dysport and if the optimal concentrations in the study were compared, i.e. Botox 25 U/ml and Dysport 100 U/ml, the ratio was 1:4.8.
The same pattern can be seen for NeuroBloc. Undiluted solution with the concentration 5000 U/ml has been used when treating muscular disorders with NeuroBloc in doses 50-100 times higher than Botox. However, when both products were diluted to the concentration 100 U/ml, the dose conversion ratio instead was 1:1.1 (Botox:NeuroBloc) in Paper I and 1:1.3 (Botox:NeuroBloc) in Paper II. For the concentration 50 U/ml, which was investigated in Paper II, the dose conversion ratio was 1:1.1 (Botox:NeuroBloc). Even though these studies are performed on sweating it is possible that the concentrations are of importance in the same way when treating muscular disorders. It would be of special interest to study NeuroBloc in lower concentrations than 5000 U/ml, which is the recommended concentration for cervical dystonia, since it has been suggested that this product is associated with a higher degree of antibody formation (59). If lower concentrations and doses can be used for this indication the risk for immunization may decrease. The optimal doses must, nevertheless, be further investigated in dose-response studies where the optimal concentrations are used.

There seems to be a limit, specific for each product, where further dilution does not increase the effect. This might be explained by the diverging formulation, in particular the amount of albumin which is an import additive since it decreases aggregation of the BTX molecules and adsorption of BTX in the vial and syringe. Furthermore, different methods are used when establishing the potency of a batch with the mouse unit assay (further described in the Products section in the Background). However, since NeuroBloc is a liquid formulation it contains supplementary additives to keep the BTX stable in solution and is therefore not fully comparable with the other three products that are formulated as powders.

Paper I and II clearly confirm that it is crucial to consider the BTX concentration in a treatment regimen, especially when switching between different products. The optimal concentration to reduce sweating varies among the products. Based on the results in Paper I and II it may be suggested that the optimal concentrations are 25 U/ml for Botox and Xeomin, approximately 100 U/ml for Dysport and 50 U/ml for NeuroBloc. However, for NeuroBloc the optimal concentration might be even lower.

**Paper III**

The median dose conversion ratio that had been used at the product switch was 1:2.3 (Botox:Dysport). After clinically judging the outcome of the initial dose, the ratio was 1:2.1 in D2-D4. There was a tendency for a more effective treatment and more adverse events after the product switch.

By the time for the follow-up the doses had been reduced and the median dose conversion ratio had decreased to 1:1.7. The adverse events reported at this point were fewer for the patients treated.
Most patients experienced no difference in treatment effect between the products at the switch. That 16% experienced a more effective treatment with Dysport could be due to the relatively higher doses that the patients received. The purpose with the study has not been to evaluate the effect for each product. It was assumed that the patients after a few treatments were near the optimal dose of BTX, i.e. a dose with no or minor adverse events and where an increased dose would add little beneficial effect, but in each individual case it is difficult to know whether the applied doses were optimal. Nevertheless, 95% of the patients did not experience an inferior treatment effect because of the used Dysport doses compared to the earlier used doses of Botox.

The median doses of BTX were relatively low in this population of patients with cervical dystonia. The reason is that the patients were not naïve dystonia patients and they received treatments not on demand but at regular 3-month intervals. With this regimen the symptoms at the time for the injections eventually disappear and the dystonic muscles will undergo atrophy after a period of therapy. Patients thus reach steady state levels with lower BTX doses required, compared to the initial treatments. The Dysport doses given since the summer of 2000 varied from 50-600 units which are, for the same reason as mentioned above, considerably lower doses than recommended by the manufacturer (250-1000 units). In a long-term follow-up of BTX A in cervical dystonia mean doses of 389±144 U had been used with satisfying results (60). The frequency of adverse events was relatively low which, according to the authors, was mainly a result of the comparatively low doses used.

In our study 24% of the patients declared adverse events of Dysport during the first year after the change from Botox; the most common ones being dysphagia, neck weakness and pain. A reason for the higher number of adverse events for Dysport at the product switch may be that the patients were treated with relatively more BTX after the change of product. The patients who reported dysphagia or neck weakness had received higher doses of Dysport than the patients without such adverse events. This is in accordance with the results from a systematic review comparing rates of dysphagia and dry mouth in studies of BTX products, where a significant positive relationship between dose and dysphagia rate was found for Dysport (61).

Only 11.3% reported adverse events at the follow-up, according to the casebook notes. Since the doses of Dysport had been lowered at this time it appears that the conversion ratio used directly after the product switch was too high. However, it is also essential to bear in mind that we, at the changeover to Dysport, asked more actively about adverse events, which could be the cause for more adverse events being reported. Many patients felt insecure when they had to change a treatment they had experienced for a long time with doses individually optimized to a level with good effect and few adverse events. When changing drug the patients were aware of the risk of
more adverse events contra insufficient treatment effect. Because of the different clinical situation during the first year using Dysport, the data is not a true indicator of a difference in frequency of adverse events between the two products, but gives important information about the risk of adverse events when changing from one product to another. When comparing the frequency of adverse events it is more appropriate to compare the reported adverse events from Botox (5.3%) with the latest data concerning the adverse events from Dysport (11.3%).

We used a lower concentration of Dysport (100 U/ml since 2002) compared to the manufacturer’s recommendation, which may decrease molecule aggregation and elevate the utilization of the BTX. Rollnik et al. have earlier shown that lower doses and lower concentrations than those recommended may give sufficient therapeutic effect; however they also added albumin to the solution (62). Based on these factors the variability in effect in studies comparing the two products using dose conversion ratios between 1:3-6 is reasonable since all of them have used dissimilar BTX concentrations and albumin content (25-29, 33, 34).

It has earlier been shown that the BTX concentration as well as the albumin content is of importance when systematically comparing different products (24). Wohlfarth et al. showed equal potency between Botox and Dysport, when both products contained the same amount of albumin and were diluted to the same BTX concentration, in an animal model and in humans (24). Quantitative electrophysiological methods were used in a mouse diaphragm model and in healthy volunteers and the dose-response curves were identical for both preparations.

In the present retrospective study, Dysport was initially used in the concentration 200 U/ml while at the follow-up 6½ years later the standard concentration for Dysport at the clinic had been changed to 100 U/ml (the same as for Botox). This might explain the decrease in the given Dysport doses at follow-up. In the clinical routine setting it is not desirable to add albumin when preparing solutions. Changing the amount of added saline can in contrast easily be made to vary the BTX concentration. It may be required to apply different BTX concentrations in clinical situations depending on the indication to treat. It also appears as the optimal BTX concentration to use is dissimilar between the products, as seen in Paper I and II.

In conclusion, the median dose conversion ratio that had been used at the product switch was 1:2.3 (Botox:Dysport). After clinically adjusting the dose, the ratio was 1:2.1 at the next three treatments. There was a tendency for a more effective treatment and more adverse events after the product switch. By the time for the follow-up the doses had been reduced and the median dose conversion ratio had decreased to 1:1.7. The adverse events reported at this point were fewer for the patients treated and thereby 1:1.7 seems to be closer to the optimal dose conversion ratio. Even though pro-
spective studies are needed to confirm the results, this report may be useful when switching between the two products.

Paper IV

The primary endpoint of this double-blind, randomized cross-over study did not show a significant difference between the treatments. This could indicate that, contrary to our belief, dose conversion ratio 1:3 may be as correct as 1:1.7. However, insufficient sample size and the cross-over design with fixed muscles injected, possibly causing carry-over effects, may have affected results. Other explanations include the lack of well-validated high-quality outcome measures for cervical dystonia (63), the high variability of dystonic symptoms with unspecific factors and a wide therapeutic range for BTX doses, rendering similar results despite the different doses (25, 64).

The estimated median TWSTRS total score was about 2 points higher for Botox (1:3) compared to Dysport 4 weeks after treatment (not statistically significant). Despite this negative result of the primary outcome and the limited generalizability because of the above-mentioned limitations, noteworthy observations were made. The results from the mixed model ANOVA at week 12 did show a statistically significant difference of 3 points in TWSTRS total between Botox (1:3) and Dysport, indicating shorter duration of effect for Botox when this ratio (low dose) was used. Furthermore, according to the post-hoc analysis concerning the patients’ assessment of the treatment effect at 12 weeks, more patients found the effect of Botox (1:3) worse compared to the Dysport treatment and Botox (1:1.7).

In all three different treatment arms the median total TWSTRS score was about 4 points lower at week 4 than at week 12 indicating a clear treatment effect (Table 6). The magnitude of improvement may not seem large, but the patients were already on stable maintenance treatment and the deterioration at week 12 reflects normal variability of symptoms. The Tsui scale and MEI also detected these changes but SF-36 and FDQ did not.

No formal statistical analyses of potential carry-over effects were included in the statistical analysis. As described by Senn (54), the carry-over effect analysis has severe limitations. A 12-week treatment interval is generally recommended, in accordance with the Summary of Products Characteristics, and patients usually report that the effect wears off after 10-12 weeks.

To account for the lack of well-validated outcome measures, we included five different instruments for evaluation in an attempt to capture different aspects of the dystonia, including physical condition and quality of life.

The range of the TWSTRS total score at baseline was wide, indicating that our sample was representative for the population. Laubis-Herrmann et al. showed that the severity of cervical dystonia at study entry did not influ-
ence therapeutic effects, measured by TWSTRS, of either preparation when comparing high and low doses of Dysport (65).

A validated objective method is well needed in this field. The relatively new and objective instrument for evaluation, MEI, has been suggested as a useful alternative measure for detecting and quantifying movement disorder in cervical dystonia (49). In the present study, the mean values of MEI indicated that this method may be used to capture treatment effects; however, the spread is wide and the more conservative use of the median value showed no substantial difference in treatment effect between the products or correlation of MEI to the other instruments for evaluation. The values of the MEI were lower after the pre-treatment evaluation, indicating a learning effect. This effect was seen before randomization and could have had a minor influence during evaluation in relation to the study treatments. The learning effect should be considered in future studies using MEI.

BTX appears to have a relatively broad therapeutic window. Different doses of the same product result in similar treatment effects in dose-response studies (25, 64). However, the frequency of adverse events is dose-dependent (64, 66), which suggests that the lowest possible dose should be used. We observed no substantial differences in adverse events between treatments (Table 8). Dose conversion ratios of 1:3 and/or 1:4 (Botox:Dysport) have been investigated in two double-blind, randomized trials treating cervical dystonia (25, 26). Ranoux et al found that both dose conversion ratios 1:3 and 1:4 (Botox:Dysport) were too high since Dysport provided a more effective treatment and longer duration of effect; furthermore the adverse events were more frequent for Dysport (25). However, Odergren et al found ratio 1:3 suitable to achieve equivalent treatment effect with a similar adverse event profile between the products (26).

In the present study, the mean dose of Dysport at inclusion was 169 units, which is relatively low compared to other studies (25, 26). Highly diluted Dysport solution (100 U/ml) has lead to use of relatively low doses of BTX at the clinic. In Paper III, dose conversion ratio 1:1.7 (Botox:Dysport) was successfully used after switching from Botox to Dysport when both products were used in the concentration 100 U/ml. This is in congruence with the results in a study observing comparable clinical improvement, measured by TWSTRS, in two groups of patients with cervical dystonia receiving low-dose (125 U/ml) or high-dose (500 U/ml) of Dysport (65). Furthermore, in Paper II, which is performed on autonomic sudomotorneurons and sweating, Dysport concentrations of 50 U/ml, 100 U/ml and 200 U/ml had a relatively enhanced sweat reducing effect compared to Dysport 500 U/ml. It thereby seems important to consider the concentration, as well as given volume, in a treatment regimen.

In conclusion the primary analysis showed that the estimated median TWSTRS total score was 1.96 points higher for Botox (1:3) compared to Dysport at week 4; however, the difference was not statistically significant.
No significant differences were seen between Botox (1:1.7) and Dysport. At week 12 a statistically significant difference in effect between Botox (1:3) and Dysport was observed, suggesting a shorter duration of effect for Botox when this ratio (low dose) was used. Furthermore, the patients’ assessments showed that ratio 1:3 resulted in suboptimal efficacy of Botox. These secondary outcome observations indicate that the dose conversion ratio between Dysport 100 U/ml and Botox 100 U/ml may be lower than 1:3, but this must be further validated in a larger patient material.
Future research

Study Botox and Xeomin in the concentration 25 U/ml in patients with hyperhidrosis, aiming to find suitable injection volumes and treatment doses when this concentration is used.

Continue the search for the optimal concentration of NeuroBloc, providing a sweat reducing peak effect. Concentrations 10 U/ml, 25 U/ml, 50 U/ml and 100 U/ml should be included.

Study NeuroBloc 50 U/ml (or lower concentration if found optimal in future studies) in patients with general hyperhidrosis and in patients with focal palmoplantar hyperhidrosis, i.e. large areas of hyperhidrosis.

Study the effect of Botox and Xeomin in the concentration 25 U/ml in patients with cervical dystonia.

Further study the dose conversion ratios 1:3 and 1:1.7 for Botox 100 U/ml and Dysport 100 U/ml in a larger group of patients with cervical dystonia.

Study the effect of NeuroBloc in low concentrations on cervical dystonia and other muscular disorders.
Sammanfattning på svenska (Summary in Swedish)

Injektioner med botulinumtoxin (BTX) är en etablerad symptomlindrande behandlingsmetod vid cervikal dystoni (ofrivilliga sammandragningar av nackmuskulatur) och hyperhidros (excessiv svettning). BTX orsakar lokal denervation genom att hindra frisättning av signalsubstansen acetylkolin och därmed dess påverkan på muskler respektive svettkörtlar.

Fyra godkända produkter innehållande BTX används vid ovan nämnda indicationer; Botox, Dysport, Xeomin och NeuroBloc. Olika studier som har jämfört effekten vid behandling av muskelsjukdom har föreslagit att Botox är 3-6 gånger potentare än Dysport, och NeuroBloc har använts i doser ca 50-100 gånger större än Botox för att uppnå likvärdig effekt. Xeomin används i samma doser som Botox. Det finns flera tänkbara anledningar till dessa skillnader, en av dem är att preparaten som studerats har varit utspädda till olika koncentrationer av BTX. Det har i litteraturen visats att graden av spädning (koncentration) kan ha betydelse för toxinets effekt.

Syftet med avhandlingsarbetet har varit att studera koncentrationens betydelse för effekten samt att jämföra Botox och Dysport vid behandling av cervikal dystoni då produkterna är utspädda till samma BTX-koncentration. Då det inte finns några tydliga riktlinjer för vilken koncentration som är bäst lämpad att använda för respektive produkt har detta undersökt systematiskt, på friska försökspersoner, för att kunna optimera behandlingen. Vid användning av lägsta effekta koncentration är det möjligt att minska dosen och därmed sänka risken för biverkningar och immunitet mot toxin. För patienter med svettningar över stora kroppsytor möjliggör låga BTX-koncentrationer att hela eller näst intill hela ytan kan behandlas eftersom en större mängd lösning för injektion erhålls vid utspädning, dvs. samma dos kan fördelas över större yta. Använda doser av de två vanligaste produktarna vid behandling av cervikal dystoni har också undersökt i en journalstudie. Dessutom har skillnad i behandlingseffekt mellan Botox och Dysport studerats för två olika så kallade dosration (dosförhållanden) då samma koncentrationer har använts. Det är viktigt att veta vilket dosratio som föreligger mellan produktarna då man behöver byta från en produkt till en annan (t.ex. vid ändrade läkemedelsavtal för landstinget). Detta för att patienterna efter bytet ska få likvärdig behandlingseffekt och för att undvika ökad frekvens av biverkningar.
Delarbete I

I arbete I undersöktes anhidrotisk (svettfri) effekt efter fristående injektioner i huden på ryggen med Botox, Dysport och NeuroBloc i tre olika koncentrationer per preparat (Xeomin var inte registrerat i Sverige vid denna tidpunkt).

Arean av anhidros, i form av vita uppklarningar, erhölls efter avtryck med ett stärkelseinnehållande kopieringspapper på jodspritpenslad rygg på försökspersonen (jod och stärkelse färgas svart vid kontakt med svett). Svettning framkallades efter kort vistelse i en bastu. Pappersavtrycken skannades och användes sedan vidare i ett datorprogram där anhidrotisk area mättes.

Den relativa anhidrotiska effekten (area per dosenhet) ökade i genomsnitt då man använde de lägsta studerade koncentrationerna av Botox och NeuroBloc. För Dysport sågs ingen skillnad mellan de använda koncentrationerna.

Delarbete II

Arbete II är en uppföljning av arbete I. Studien utfördes på samma sätt med injektioner i huden på ryggen följt av jod-stärkelsetest och areamätning i datorprogram. Fyra olika koncentrationer av Botox, Dysport, Xeomin och NeuroBloc studerades. Koncentrationerna valdes utifrån resultaten i arbete I. I denna studie undersöktes även hypohidrotisk (svettreducerad) area samt longitudinell effekt.

Resultaten överensstämde väl med studie I och visade att den relativa anhidrotiska effekten (area per dosenhet) i genomsnitt ökade med lägre använda koncentrationer. För Botox och Xeomin hittades en optimal koncentration, dvs en tydlig gräns för när spädning inte längre gav ytterligare relativ effekt. För Dysport sågs ingen uttrycklig skillnad mellan de tre lägsta använda koncentrationerna men dessa tre gav större area per dosenhet jämfört med den högsta koncentrationen. För NeuroBloc gav den lägsta använda koncentrationen störst area per dosenhet men optimal koncentration kan vara lägre, vilket måste studeras vidare.

Delarbete III

I arbete III undersöktes vilka doser av Botox och Dysport som hade använts vid cervikal dystoni (n=75) i samband med produktbyte på neurologkliniken, Akademiska sjukhuset. Det är en retrospektiv journalstudie som presenterar använda medianterader av Botox före byte till Dysport samt mediandoser av Dysport direkt efter bytet samt ca 6 år senare. Förutom läkemedelsdoser
presenteras biverkningar och patienternas skattning av behandlingseffekt av respektive produkt.

Studien visade att det initialt högre dosratiot (1:2,3, Botox:Dysport) vid bytet gav mer biverkningar och en tendens till bättre effekt av Dysport och att mediansdosen hade sänkts vid uppföljningen 6 år senare (till dosratio, 1:1,7) med mindre rapporterade biverkningar som följd.

Delarbete IV

Arbete IV är designat med utgångspunkt från arbete III. I studien undersöktes skillnad i behandlingseffekt mellan Botox och Dysport för två olika dosration (1:3 och 1:1,7, Botox:Dysport) hos patienter med cervikal dystoni (n=46). Samma koncentration (100 enheter/ml) användes för produktomma. Studien var utformad som en cross-over där alla patienter, blindat, fick 3 olika behandlingar (Botox i två olika doser samt Dysport som kontrollbehandling). Effekten utvärderades 4 respektive 12 veckor efter varje behandling med hjälp av fem olika utvärderingsinstrument för att inkludera fysiskt tillstånd, mental hälsa och livskvalitet.

Det var ingen statistiskt signifikant skillnad mellan Botox (1:3) och Dysport, inte heller mellan Botox (1:1,7) och Dysport fyra veckor efter behandling. En del av de sekundära analyserna indikerade dock att Botox i dosratio 1:3 inte gav optimal effekt men detta måste studeras vidare då studien inte lyckades rekrytera tillräckligt många patienter för att nå statistisk styrka.
My sincere gratitude goes to all the **patients and healthy volunteers** who have given their time to participate in these studies.

I would also like to express my genuine thanks to the following persons:

**My supervisors, Dag Nyholm and Carl Swartling**, for excellent tutoring, collaboration and support in the kindest way. Your different areas of expertise have been a good combination which has helped me forward in my work.

**Carl Swartling**, my co-supervisor, who first introduced me to the subject in 2004 and has accompanied me since then, supplying me with a great amount of knowledge within the field of hyperhidrosis. Your sense of humor is one of a kind!

**Dag Nyholm**, my principal supervisor, who has provided brilliant guidance during the studies concerning cervical dystonia and has taught me very much about the regulatory work that comes with clinical studies. I’m impressed by your ability to get so many things done!

**Hans Naver**, my former supervisor, a central person in this thesis work who has unique experience of using botulinum toxin in the treatment of hyperhidrosis and cervical dystonia. I’m grateful for the warm and kind supervision you have provided throughout these years.

**Håkan Askmark**, my former supervisor, for support in the beginning of the research program.

**Gun Schönnings**, for always being so kind and helpful with administrative tasks and for creating a friendly atmosphere at work.

**Anders Johansson**, for showing me the clinical aspects of cervical dystonia and helping me understand more about the disease and the methodology around the treatment. Thank you for all encouraging and rewarding discussions during the trial and the writing of our manuscript.
Lena Zetterberg, who enthusiastically has taught me almost everything I know about the instruments used when evaluating cervical dystonia. Thank you for inspiring and very relevant discussions in this subject during our work together and also for being so thoughtful towards me.

Co-authors: Joachim Burman, Mattias Karlqvist and Maria Bertilsson, for nice collaboration during the studies.

Olga Tarassova, Ylva Åkerblom, Gunilla Elmgren Frykberg and Carolina Färnstrand, for nice collaboration at the gait laboratory.

Uppsala Clinical Research Center (UCR), for statistical support.

All present and former working staff at the outpatient clinic of Neurology (neuromottagningen) and the department of Neuroscience, Neurology (läkarrumskorridoren), for nice collaboration during the clinical trials but most of all for friendship and many laughs in the lunch room and at the “fika” in the corridor.

My office roommates: Jimmy Sundblom, Katherina Nousia and Johan Virhammar, for refreshing conversations between work.

Peter Mattsson, for letting me borrow the mirror.

Tack till min familj och mina vänner för trivsamt och glatt sällskap på fritiden.

Särskilt tack till:

Linda Gimle, Åsa Sandgren och Therese Mattsson för att ni alltid finns där även om det ibland går lång tid mellan gångerna.

Simon Rystedt, min svåger, för Photoshop-support under avhandlingsarbetet och för många roliga pratstunder under tiden du bodde hos oss. Saknar dig hemma i Sverige!

Marie Karlsson och Kerstin Karlsson, mina systrar, för vänskap och alla glada minnen under min uppväxt. Tack Marie för din hjälp med avhandlingsarbetet och många bra förslag på orkestrar!

Kerstin Rystedt och Alf Johansson, mina svärföräldrar, för ovärderligt stöd med barnpassning och för att ni alltid är så snälla och tillmötesgående.

Einar Rystedt, min älskade man, för kärlek, vänskap och mycket skratt. Tack för all hjälp med Photoshop och alla konstruktiva kommentarer på avhandlingsarbetet. Tack för att du stöttar mig och säger uppmuntrande ord när jag behöver det som mest!

Viola Rystedt, min älskade dotter, som får mig att le varje dag!
References


38. Swartling C. Botulinum toxin in the treatment of focal hyperhidrosis and dyshidrotic hand dermatitis. Uppsala: Department of Medical Sciences, Section of Dermatology and Venereology, Uppsala University; 2002.


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine.