Available online at

ScienceDirect
www.sciencedirect.com

Elsevier Masson France

EM|consulte
www.em-consulte.com

Physical & Rehabilitation Medicine

SOFMER
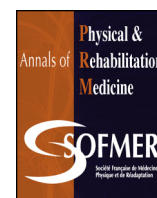
Literature review

Muscle structure and stiffness assessment after botulinum toxin type A injection. A systematic review

CrossMark

L. Mathevon [a,*], F. Michel [b], P. Decavel [b], B. Fernandez [a], B. Parratte [c], P. Calmels [a]

[a] Service de médecine physique et de réadaptation, laboratoire de physiologie de l'exercice, université Jean-Monnet, CHU de Saint-Étienne, Saint-Étienne, France
[b] Service de médecine physique et de réadaptation, université de Franche-Comté, CHU de Besançon, Besançon, France
[c] Service de médecine physique et de réadaptation, laboratoire d'anatomie, université de Franche-Comté, CHU de Besançon, Besançon, France

ARTICLE INFO

ABSTRACT

Background: Botulinum toxin type A manages spasticity disorders in neurological central diseases. Some studies have reported that it might induce muscle changes.
Methods: We present a literature review abiding by the PRISMA statement guidelines. The purpose was to explore the structural and passive biomechanical muscle properties after botulinum toxin type A injections in healthy and spastic limb muscles, on animals and humans, as well as methods for evaluating these properties. We searched the PubMed and Cochrane Library databases using the following keywords: "Botulinum toxin" AND ("muscle structure" OR "muscle atrophy") and, "Botulinum toxin" AND "muscle elasticity".
Results: From the 228 initially identified articles, 21 articles were included. Histological analyses were performed, especially on animals. A neurogenic atrophy systematically occurred. In humans, one year after a single injection, the histological recovery remained incomplete. Furthermore, 2D ultrasound analyses showed a reduction of the gastrocnemius thickness and pennation angle. MRI volumetric analysis evidenced muscular atrophy six months or one year after a single injection. Passive muscle stiffness depends on these structural changes. On the short term, the biomechanical analysis showed an elastic modulus increase in animals whereas no change was recorded in humans. On the short term, ultrasound elastography imaging showed a decreased elastic modulus.
Discussion: To date, few data are available, but all show a structural and mechanical muscle impact post injections, specifically muscle atrophy which can linger over time. Further studies are necessary to validate this element, and the possibility of change must be taken into account particularly with repeated injections. Thus, in clinical practice, 2D ultrasound and ultrasound elastography are two non-invasive techniques that will help physicians to develop an efficient long term monitoring.

© 2015 Elsevier Masson SAS. All rights reserved.

1. Introduction

The spastic paretic muscle is subject to structural changes compared to healthy skeletal muscles. These changes are independent from the initial etiology of the neurological lesion. The literature describes an increased variability of the size and type of muscle fibers, decreased numbers of sarcomeres, proliferation of the extracellular matrix (ECM) with an increased collagen concentration [1,2]. When the first motor neuron is affected, muscle retraction can get settled under the influence of two main factors. The disuse of the

paretic muscle or "functional immobilization", which initiates muscle atrophy and reduces the number of sarcomeres by a disequilibrium of the protein-proteolysis synthesis balance in favor of the proteolysis [3,4]. Chronic muscle hyperactivity maintains the muscle in a short position, reducing the longitudinal tension [5]. Thus, the shortening of the muscle fibers and accumulation of conjunctive tissue are responsible of these changes in biomechanical viscoelastic properties of the muscles, with a decreased passive extension capacity [3–6].

Muscle changes contribute to functional impairments. Gait speed and step length have been significantly correlated to passive mechanical properties of the plantar flexor muscles, determined by the measure of the passive torque/joint angle ratio of the talocrural joint [7]. For Dietz and Sinkjaer, the spastic muscle at rest is

submitted to an overexcitability of the alpha motor neuron (presynaptic inhibition and increased activity of type Ia ascending fibers) with little changes during voluntary contractions. Thus, hyperreflexia is hardly involved in pathologic spastic movements [6].

Injections of botulinum toxin type A (BoNtA) are a first-line therapeutic method to treat focal spasticity [8]). Their action is triggered by the fixation on the SNARE proteins and inhibited release of acetylcholine (ACh) from the presynaptic terminals. The therapeutic objective is to decrease reflex muscle overactivity and fight muscle hypertonia. The functional benefit will affect gait patterns and movement amplitude [9–12]. However, the impact of BoNtA injections on muscle structure and the stretching capacity of muscle tissue have rarely been reported in the literature. The challenge is to differentiate the consequences related to spastic paresis from those linked to the injections of botulinum toxin. We propose a review of the literature with the following objectives:

- analyze changes in the structure and stiffness of muscle tissue described after an injection of botulinum toxin in one muscle of the limbs;
- discuss the evaluation methods used.

2. Methodology

A systematic review of the literature was conducted abiding by the PRISMA recommendations (www.prisma-statement.org). We searched the Pubmed and Cochrane Library databases, using the following keywords

"Botulinum toxin" AND ("muscle structure" OR "muscle atrophy") and, "Botulinum toxin" AND "muscle elasticity". Articles stemming from this research were independently put aside by 2 authors (LM and BP) and were then evaluated. Articles were kept if they met the following criteria:

- the study focused on the analysis of a striated skeletal muscle of the limbs, paretic spastic or healthy (to differentiate changes related to spastic paresis), in men and humans;
- the study analyzed the consequences of injections of botulinum toxin on muscle structure and/or muscle tissue stiffness;
- evaluation methods in the fields of histological, mechanical and medical imaging analyses were described;
- the full manuscripts were published in English between 1990 and October 2014. References of the articles included were used to eventually complete the selection. In case of disagreement, a decision was taken after further discussion.

The methodological quality of the articles was evaluated using a specific scale developed based on the STROBE (Strengthening the Reporting of Observational studies in Epidemiology) principles [13]. Each item was categorized, and the maximum global score is 28 (Table 1).

3. Results

3.1. Selection of the studies

In all, 228 articles were initially identified (Fig. 1). Thirty-two articles were deemed relevant after reading the titles and abstracts. Twenty-one were included in the review (11 were excluded because they did not meet the selection criteria). Fourteen studies focused on the structural analysis, 3 studies on muscle stiffness analysis and 4 studies on both structure and stiffness analysis. Overall, there were very few studies, with often a

Table 1
Quality analysis form used in the systematic review.

Q1	Is there in the abstract an explanation of what was done and found?
Q2	Is the scientific context clearly explained?
Q3	Are the objectives clearly stated?
Q4	Is the sampling size indicated?
Q5	If yes, is the sampling size statistically justified?
Q6	Are the characteristics of the subjects (height, weight, sex, healthy or pathologic subject) described?
Q7	What is the design of the study? (0: retrospective study; 1: case study; 2: prospective study).
Q8	Is there a control group? (0: no, 1: contralateral member or non-randomized control group, 2: randomized control group).
Q9	How long is the follow up? (0: ≤ 1month; 1 ≤ 6 month; 2 ≥ 1 year)
Q10	Is the reliability of the evaluation method clearly described?
Q11	Are the results interpretable?
Q12	Are the limitations of the study discussed?
Q13	Is the conclusion clearly stated?

0: no description; 1: limited description; 2: good description.

restricted population sample (from 1 to 56 patients in men) and various exploration methods.

3.2. Quality of the reviewed articles

The quality of the reviewed articles is summed up in Table 2. It is highly variable. Most studies were prospective ones, except for 2 case studies [14,15] and a retrospective study [16]. The descriptive quality of the experimental protocol, results as well as their interpretation and conclusion was adequate in most studies. The reproducibility of evaluation method was rarely described. No study proposed sample size calculations. The follow-up duration was quite short in most studies (≤ 3 months).
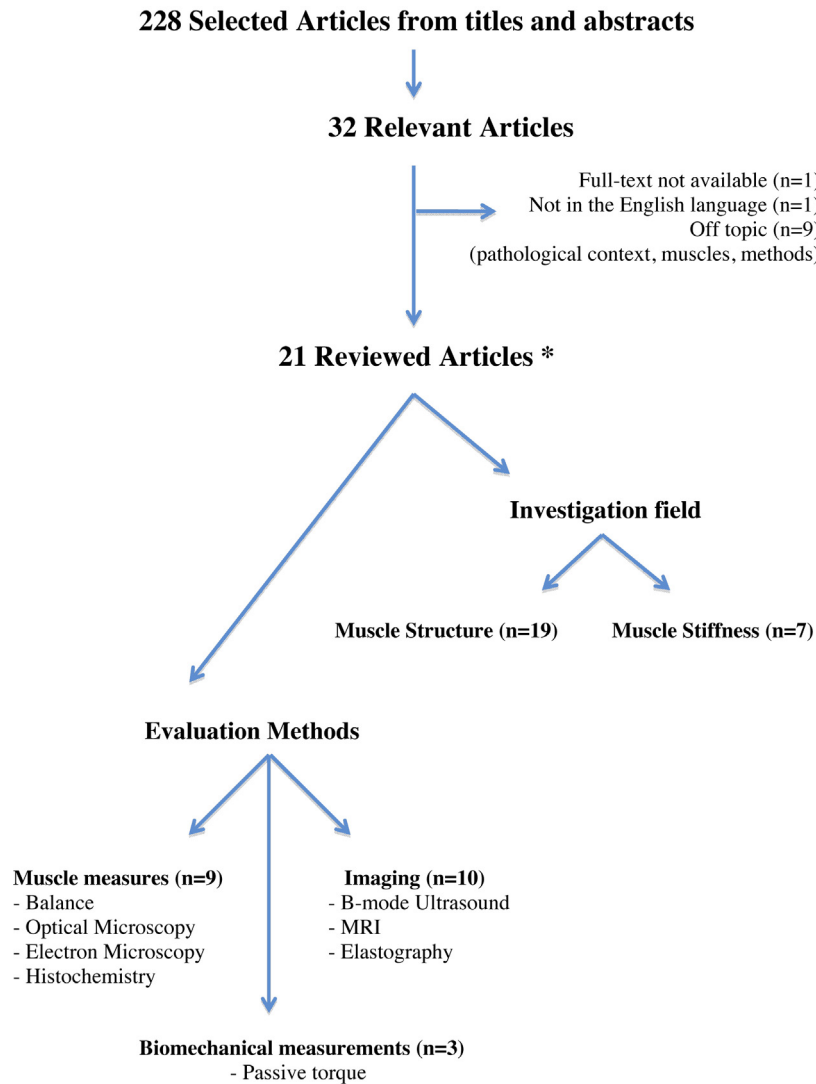
3.3. Literature analysis (Table 3)

The methodological variability among the small number of studies, made it mandatory to conduct an evaluation based on changes in muscle structure and passive mechanical properties and according to the exploration techniques used.

3.3.1. Structural changes

Analysis via muscle samples in animals, muscle mass analysis based on post-mortem dissection reports post-injection atrophy [17–24]. It occurs from 1 week [22,24] to 4 months [24] after one injection, with a dose effect. A dose twice as important (6 U Botox/kg versus 3 U Botox/kg) reduces the delay of onset to one week (versus 4) [23]. After one single injection, the intensity of the loss in muscle mass varied from 30 to 60% (dose effects, muscles and animal injected) according to the various studies [17–24]. The recovery was highlighted at 4 months [23] or 1 year [24]. The recovery was incomplete, in the range of 90% recovery of the initial muscle mass. Following repeated monthly injections, Fortuna et al. [19] brought up the possibility of a ceiling effect of the atrophy after the third injection, around 60%. At one year, results showed that repeating an injection at 6 months majors muscle atrophy and in a dose-dependent manner.

Histological slides were used to analyze muscle fibers [17–19, 21,22,24,25], via optical (OM) [17] and electronic microscopy (EM) [22,24]. A precise analysis of contractile proteins [19,21] (auto-mated measurement technique: Mat Lab Program) [19] was conducted which also included the composition of myosin heavy chains, titin molecular mass (gel electrophoresis), extracellular matrix and amount of collagen (hydroxyproline assay) [25]. After one single injection, the structure of the sarcomeres is altered with a misalignment of Z-lines. The initial structure was restituted at 6 months [24]. Changes in contractile proteins were observed.

228 Selected Articles from titles and abstracts

↓

32 Relevant Articles

→ Full-text not available (n=1)
Not in the English language (n=1)
Off topic (n=9)
(pathological context, muscles, methods)

↓

21 Reviewed Articles *

Investigation field

Muscle Structure (n=19)   **Muscle Stiffness (n=7)**

Evaluation Methods

Muscle measures (n=9)
- Balance
- Optical Microscopy
- Electron Microscopy
- Histochemistry

Imaging (n=10)
- B-mode Ultrasound
- MRI
- Elastography

Biomechanical measurements (n=3)
- Passive torque

* A common study in humans and animals. Some articles are referring to multiple methods
and fields of investigation.

Fig. 1. Selection diagram of the reviewed articles. * A common study in humans and animals. Some articles are referring to multiple methods and fields of investigation.

Table 2
Quality assessment.

	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Total (max = 28)
Alhusaini et al. (2011)	2	2	1	2	2	2	2	0	1	1	2	2	2	21
Boyaci et al. (2014)	2	2	1	2	0	2	2	1	0	0	0	2	1	15
Choi et al. (2007)	2	1	1	2	0	2	2	1	0	1	1	0	2	15
Dodd et al. (2005)	2	2	2	0	0	1	2	2	1	1	1	0	2	16
Fortuna et al. (2011)	2	2	2	2	0	2	2	2	1	1	1	1	2	20
Frick et al. (2007)	2	2	1	2	0	2	2	2	1	1	2	1	2	20
Haubruck et al. (2012)	2	2	2	2	0	2	2	2	0	1	2	2	2	21
Kwon et al. (2012)	1	1	1	2	0	2	1	0	0	0	1	0	2	11
Legerlotz et al. (2009)	2	2	2	2	0	2	2	2	0	1	2	0	2	19
Ma et al. (2004)	2	2	2	2	0	2	2	2	2	2	2	1	2	23
Park and Kwon (2012)	2	2	1	2	0	2	2	0	0	1	1	2	2	17
Picelli et al. (2012)	2	2	2	2	1	1	2	0	0	1	1	2	2	18
Schroeder et al. (2009)	2	2	2	2	0	1	2	1	2	1	2	0	2	19
Shaikh et al. (2014)	1	2	2	2	0	2	0	2	1	1	1	1	2	17
Stone et al. (2011)	2	2	1	2	0	1	2	0	1	1	1	1	2	16
Thacker et al. (2012)	2	2	1	2	0	2	2	1	0	1	2	1	2	18
Tok et al. (2011)	2	1	2	2	0	2	2	0	1	0	1	1	2	16
Tsai et al. (2010)	2	2	1	2	0	1	2	1	2	1	1	1	2	18
Van Campenhout et al. (2013)	2	2	2	2	0	1	2	0	1	2	2	2	1	19
Vasilescu et al. (2010)	1	2	1	2	0	2	1	0	0	0	1	0	1	11
William et al. (2013)	2	2	1	2	0	2	0	0	1	2	2	2	2	18

Table 3
Summary table of the results.

Author Year	Human/Animal	Control group	Age	Population (number)	Neurological Condition	Muscle system	BoNtA Number of injections and dose	Measurement tool	Study criteria	Changes	First–Last evaluation	First changes	Recovery
Alhusaini et al., 2011	Human	No	4 to 10 years	16	CP	TS+TA+TCJ unit	Botox, 1, ?	Dynamometer–Potentiometer	TS+TA +TCJ unit stiffness	None	6 weeks–6 weeks	None	?
Boyaci et al., 2014	Human	Yes	Avg; 49 ± 16 months	16	CP	GM, GL, So	Botox, 1, 1,5U/kg in GM	B-mode US	Muscle thickness	None in injected GM Increase in GL, Soleus	1week pre-BoNtA–4 weeks post-BoNtA	4 weeks	?
								Sonoelastography	Strain ratio / Red Pixel intensity	Decrease in injected GM and in GL / Decrease in injected GM and in GL			
Choi et al., 2007	Rat	Yes	Mature, 4 year	80	Healthy	RF	? 4 groups 0, 1, 3, 9 ng/kg/day, daily for 4 weeks	Balance	Body weight	Dose-dependent weight loss	D1–4 weeks	Dose-dependent	No
								Histology	Muscle structure	Decrease of muscle fiber diameter / Increase of nuclei number / Increase of collagen / Increase of adipocytes			
Dodd et al., 2005	Rat	Yes + contralateral limb	Mature, 4 months	?	Healthy	TS	Dysport, 1 4 groups: 3 U, 6 U, 12 U, 18 U	Balance	Muscle mass	Decrease	D1–D67	D67	No
								Histochemistry	MHC isoforms	Decrease MCH IIx → IIa et I			
Fortuna et al., 2011	Rat	Yes	Mature, 1 year	20	Healthy	RF, VL, VM	Botox, monthly, 3,5 U/kg	Balance	Muscle mass	Decrease	1 months–6 months	1 month	?
								Histology	Contractile proteins %			3 months	
Frick et al., 2007	Rat	Yes + contralateral limb	Mature	30	Healthy	TA	Botox, 1 3 groups: 0,625 U, 2,5 U, 10 U	Balance	Muscle mass	Dose-dependent decrease	128 days–128 days	128 days	?
Haubruck et al., 2012	Rat	Yes	Mature	36	Healthy	GCM+TA + calcaneus unit	?, 1, 6 U/kg	Dynamometer+ lenght measure	GCM+TA + calcaneus unit stiffness	Decrease	8 days–8 days	8 days	?
Kwon et al., 2012	Human	Yes	28 years	1	CP	GCM, So	Botox, 1, 20 U	B-mode US	Muscle thickness	Increase	4 weeks–4 weeks	4 weeks	?
								Sonoelastography	Elastic modulus with color maps	Softer muscles			
Legerlotz et al., 2009	Rat	Yes	Juvenile, 4 weeks	30	Healthy	GCM	Botox, 1, 13 U/kg	Balance	Muscle mass	Decrease	3 weeks–3 weeks	3 weeks	?
								Histochemistry	MHC isoforms	MHCIIb → MHCIIx or IIa			
Ma et al. 2004	Rat	Yes	Juvenile, 1 month	34	Healthy	GM, GL	?, 1 6 U/kg	Gel Electrophoresis	Titin content	Decrease	1 week–1 year	1 week	6 months
								Balance	Muscle mass	Decrease			
								Histology: ME	NMJ morphometry: width	Increase		2 months	1 year
Park et Kwon, 2012	Human	No	Avg; 57 ± 22 months	17	CP	GM, GL	Botox, 1, 20 U/muscle	Sonoelastography	RTS score / Red pixel intensity	Decrease / Decrease	4 weeks	4 weeks	?
Picelli et al., 2012	Human	No	Avg; 59 ± 14 ans	56	Stroke	GM, GL	?, 1, 250 U/GCM	B-mode US	Muscle echogenicity, Heckmatt Scale	No echogenicity changes Injections less effective depending on echogenicity	4 weeks–4 weeks	4 weeks	?
Schroeder et al., 2009	Human	Contralateral limb	31 and 47 years	2	Healthy	GL	Xeomin, 1, 75 U	MRI	Muscle signal / Muscle cross-sectional area	High signal intensity in STIR sequence / Decrease	3 months–12 months	3 months	No

Table 3 (*Continued*)

Author Year	Human/Animal	Control group	Age	Population (number)	Neurological Condition	Muscle system	BoNtA Number of injections and dose	Measurement tool	Study criteria	Changes	First–Last evaluation	First changes	Recovery
								OM	Muscle structure	Neurogenic atrophy Reinnervation			
Shaikh et al., 2014	Human	Yes	Avg: 47 years (min: 26–max: 63)	12	Piriformis syndrome	Piriformis	Botox, inconstant injections number, 100 U	EM MRI	Muscle ultrastructure Maximal muscle thickness	Decrease	Inconstant Avg: 7,3 ± 5,2 months post-injection	?	No
									Muscle volume Fatty infiltration	Decrease Increase			
Stone et al., 2011	Mouse	No	?	140	Healthy	GM, GL	Botox, 1, inconstant doses and volumes	Balance	Muscle mass	Dose- and volume-dependant decrease	4 weeks–12 weeks	4 weeks	12 weeks
Thacker et al., 2012	Rat	Contralateral limb	Mature	24	Healthy	TA	Botox, 1, 6 U/kg	Dynamometer + Length measure (muscle fibers) Histology	Elastic modulus Collagen content	Increase Increase	1 month–1 month	1 month	?
Tok et al., 2011	Human	Contralateral limb	Avg: 55 ± 14 years	26	Stroke	GM, GL	?, 1, ?	B-mode US	Pennation angle	Decrease	10 days–2 months	2 months	?
									Fascicular lenght Muscle thickness Muscle compressibility	Increase Decrease None			
Tsai et al., 2010	Human	No	?	5	Calf asymetry	TS	Botox, 1, 20 ng calf	Meter	Maximal calf circumference	Decrease	4 weeks–26 weeks	4 weeks	26 weeks
	Rat	Yes	?	5	Healthy	GCM	3 groups: 1,5 ng/kg 6 months repeated 1,5 ng/kg 6 months repeated 1 ng/kg	EM	Muscle ultrastucture	Sarcomere distorsion	1 week–52 weeks	1 week	26 weeks
								Balance	Muscle mass	Decrease			
Van Campenhout et al., 2012	Human	No	Avg: 12 years	7	CP	Proximal psoas Distal psoas	Botox, 1, 2 U/kg/psoas	MRI	Muscle volume	Decerase (proximal injected psoas) None (distal injected psoas)	2 months–6 months	2 months	No
Vasilescu et al., 2010	Human	Contralateral limb	3–10 years	7	CP	Inconstant	?	B-mode US	Muscle echogenicity Aponevrosis echogenicity, Diamètre musculaire	No description	?	?	?
								Sonoelastography	Elasticity pattern with color map	Softer muscles			
William et al., 2013	Human	No	5–11 years	15	CP	GCM, So	Botox, 1, inconstant doses	MRI	Muscle volume	Decrease in injected muscles Increase in soleus muscle	5 weeks	5 weeks	?

Avg: average; CP: cerebral palsy; TS: triceps surae; TA: tibialis anterior; TCJ: talocrural joint; GM: gastromedialis; GL: gastrolateralis; GCM: gastrocnemius muscle; So: soleus; RF: rectus femoris; VS: vastus lateralis; VM: vastus medialis; US: ultrasonography; EM: electron microscopy; OM: optical microscopy; MHC: myosin heavy chains; NMJ: neuromuscular junction.

After one injection, the percentage of fast twitch Type IIb fibers decreases in favor of intermediate Type IIa fibers and slow twitch Type I fibers, in the gastrocnemius muscles [18,21] and the tibialis anterior muscle [25]. In monthly injections, the quantity of myosin heavy chains (MHC) decreases after the 3rd month (in the range of 70% of surface MHC on the rectus femorus and vastus lateralis, vs. > 90% before the injection as evidenced on histological slides) [19]. It decreases again down to about 40% at 6 months, on the vastus lateralis [19]. Finally, 1 month after one injection, the amount of collagen in the muscle increases (in the order of 40%), as well as the expression of the titin protein, the most abundant elastic protein in the muscle fiber [21,25].

In humans, only the work of Schroeder et al. [26] reported histological data based on muscle biopsies. One year after one single injection of 75 U Xeomin in the lateral gastrocnemius muscle, the authors reported changes in the muscle structure [26]. In optical microscopy, the surface of muscle fibers is reduced by 24%, muscle fibers have an angular shape [27], and the atrophy is compensated by a process of fibrosis. Under electronic microscopy, authors evidenced a negative progression of neuro-muscular junctions, with an increase of the space located between nerve endings and motor plates. A decrease in the number of crests of the synaptic folds, where RnAch accumulates, was noted.

3.3.2. Medical imaging analysis

2D ultrasound was used in 4 studies [15,28–30], 2 in children with cerebral palsy [15,28] and 2 in post-stroke hemiplegic patients [29,30]. This technique allows a reproducible evaluation of the muscle by measuring muscle thickness, a section of muscle surface and the pennation angle [21]. Tok et al. [30] and Picelli et al. [29] respectively reported results on 56 and 26 hemiplegic patients (chronic phase > 1 year) by making a comparison with the contralateral limb which did not receive any injections. The ultrasound analysis was conducted 1 to 2 months after a single injection in the gastrocnemius muscles with commonly-used doses. Tok et al. excluded patients who benefited from injections within the previous year [30]. They reported a decrease in muscle thickness and for the pennation angle as well as an increase in the muscle fascicle length [30]. Picelli et al. [29] did not report any changes in muscle echo intensity after injection. They considered that the efficacy of the injections was diminished in spastic muscles with increased echo intensity, evidencing muscular degeneration (Heckmatt III-IV scale). Boyaci et al. [28] in 16 children with cerebral palsy and Kwon et al. [15] on one single patient, reported increased medial gastrocnemius thickness 4 weeks after one single injection (in the range of 2 mm). However, the injection was performed with lower doses than those usually used and children had previously benefited from an intensive rehabilitation program including muscle strengthening.

Magnetic Resonance Imaging (MRI) helps refine the level of muscle atrophy by quantifying muscle volume and analyzing the signal of fatty tissue involution. According to Schroeder et al. [26], Van Campenhout et al. [33], and William et al. [34], after a single injection of botulinum toxin, muscle atrophy sets in (from 4 to 30%). This atrophy lingers from 6 months to 1 year after an injection in healthy subjects [26], and in diplegic children [33]. For Van Campenhout et al., the proximal injection of the psoas fascia of the iliopsoas muscle induces a 20% reduction in muscle volume at 2 months [33] whereas, a distal injection was not correlated to atrophy.

3.3.3. Changes in the passive mechanical properties of the muscle

We looked for changes in the passive stiffness of the muscular system (ratio between the variation in muscle length and the stretching force). It is most often quantified based on Young's modulus (E) determined by the ratio stress/strain ($E = \sigma/\varepsilon$, in MPa with σ = stress in MPa and ε: $L - L0/L0$ = strain).

Two studies on animal models reported an in-vitro analysis on a healthy muscle [25,35]. The analysis was performed at the scale of the muscle (gastrocnemius, calcaneal tendon, calcaneus) related to its lever arm: [35], or at the scale of a fiber bundle of the anterior tibialis muscle [25]. Calculation of the elastic modulus was performed by creating stretching and recording the changes in the length of the muscle-tendon unit or muscle fibers and passive strain necessary for displacement. Haubruck et al. [35], showed that 8 days after an injection there was a significant reduction of stiffness in the range of 30%. For Thacker et al. [25], one month after an injection on a single muscle fiber, the elastic modulus decreased by 15% and so did the muscle fiber. Furthermore, the passive elastic modulus of fiber bundles doubled after one injection. The variable difference between a unique muscle fiber and fiber bundles can be explained by the accumulation of intermyofibrillar collagen.

Five studies analyzed the viscoelastic properties of spastic muscles, including 4 studies on children with cerebral palsy after injection of BoNtA [14,15,28,36,37]. Alhusaini et al. [36] assessed the stiffness of the triceps surae–calcaneal tendon–talocrural joint unit, by measuring the passive torque during passive rhythmic rotation of the ankle at slow speed (60°/s). They did not evidence any significant changes in stiffness 6 weeks post injection. The other studies used elastography imaging [14,15,28,37]. Its principle resides in measuring the strain induced by the compression of biological tissues. The Shear Wave Elastography (SWE) technique implies the production of shear waves through the tissues, thus generating a strain on the tissue. The elasticity of muscle tissue is represented by color mapping [14,15,28,37] and quantified by measuring either the strain ratio or the elastic modulus. When the elastic modulus is greater, the muscle becomes stiffer. Four weeks after an injection in the medial gastrocnemius muscle, there is a decrease in muscle stiffness related to a reduction of spasticity [15,28,37]. Studies by Boyaci et al. and Park et al. [28,37] associate the effect of the botulinum toxin injection to an intensive rehabilitation program (stretching and muscle strengthening exercises).

The study of muscle stiffness is quite complex. It must be in accordance with the system of analysis used: muscle fiber, muscle, muscle-tendon unit, muscle-tendon-joint unit, and the evaluation method. In children with cerebral palsy, a few weeks after an injection of botulinum toxin, the stiffness of the system: triceps surae–calcaneal tendon–talocrural joint, measured using the ratio passive torque/angle joint was not modified [36]. However, the stiffness of the medial gastrocnemius muscle, measured via SWE, had decreased [15,28,37].

4. Discussion

Looking for structural and mechanical changes in the spastic muscle after injection of botulinum toxin has rarely been reported in the literature, in humans and animals alike, and the few studies that did focus on this topic had very different analytic methods. However, the stakes of such changes are very important: being aware, on a structural level, of the muscular deterioration induced by botulinum toxin; deducing the changes in tissue stiffness in regards to the system of analysis chosen; and implementing in humans reliable evaluation modalities for both muscle structure and stiffness.

In fact, on a structural level, we can report that in animals, one single injection of BoNtA induces muscle atrophy that lingers for at least one year, with loss of muscle mass. Repeated injections major this atrophy and increase its duration with a dose-effect and frequency of the injections [19,24]. The transition of myosin heavy chains (MHC) towards slower phenotypes [18,21,25], might change the conditions of the muscle fibers' dynamic contraction

[38]. For gastrocnemius muscles that have a faster contraction profile and are often injected, this decrease in contractile proteins induces a decrease in the maximum strength, lingering more than one month post injection, it is proportional to the dose injected [18,39] and has an impact on the shortening speed of muscle fibers. In humans, these data cannot be fully extrapolated. Only the study by Schroeder et al., in healthy lateral gastrocnemius muscles, unveils classic signs of neurogenic atrophy [26].

Based on a non-invasive exploration via 2D ultrasound, the measurements of muscle thickness and pennation angle at the level of the gastrocnemius muscles are reproducible at rest in healthy subjects, children [40], older adults [31] and in post-stroke hemiplegic patients [32]. This requires abiding by a standardized procedure for the location of ultrasound landmark measurements. However, the articles in this literature review presenting the architectural consequences on muscles of botulinum toxin injections did not describe any standardized measurement procedures [29,30]. The following settings and procedure must be respected to meet reproducibility and sensitivity criteria: 2D ultrasound machine with a probe adapted for muscle analysis (linear probe with a 6-15 Hz frequency); experienced operator with a solid knowledge of muscle anatomy, including the fascial organization of muscles to evaluate changes in muscle architecture via ultrasound; finally a validated institutional reproducible procedure of ultrasound anatomical landmarks and measurements done. Furthermore, it has been reported in 2D ultrasound that when the muscle has a high signal intensity and a loss of fascicle-like organization of the muscles fibers, there is a greater fatty infiltration muscle fibrosis [41]. This latter evaluation is strictly qualitative and depends on a wide inter-individual echogenicity, especially according to the thickness of the subcutaneous fat pedicle. The use of MRI in muscle analysis has rarely been reported in this context of post botulinum toxin injection [26,33,34], whereas the technique allows an interesting analysis of the denervated muscle [42].

Muscle atrophy has been evidenced, but its intensity, duration, reversible nature, or even its compensation by other muscles remains unknown. According to the study by William et al. [34], among a population of children with cerebral palsy, the volume of the non-injected soleus muscle was increased (4%) suggesting a compensating mechanism within the triceps surae muscle.

Studies on musculoskeletal stiffness after injection of botulinum toxin in humans and animals were conducted with very different protocols, all using variable criteria. Thus, the resulting data are very heterogeneous and can sometimes seem contradictory. There has not been an assessment of the consequences of injections on muscle stiffness beyond 6 weeks. However, patients who benefit from a treatment with botulinum toxin receive repeated injections several times a year, for a long period of time. The concept of muscle stiffness is related to its behavior as a viscoelastic environment. In fact, in the case of the spastic muscle, stiffness can be refined and assessed according to the analytic approach. In a clinical approach, it is the limitation of the joint range of movement when the muscle is subjected to passive stretching (stretching hyperreflexia or tendon-muscle retraction). In a biomechanical approach, the study refers to a model analyzing the elastic structures of the muscle-tendon system, such as the biomechanical muscle model of Hill [43] that takes into account the contractile element (CE) and two non-linear spring elements, one in series (SE) and another in parallel (PE). The latter yields information on viscoelastic properties at rest. In humans, the evaluation of passive muscle stiffness is possible on a "relatively" isolated muscle using passive movements [44]. The relationship passive torque/joint angle is based on the measurement of the force exerted to mobilize the joint under different angles. This relationship is not a linear one and maximum torque can vary depending on the given joint [45] due to the variable quantity of conjunctive tissue in the muscle groups. This method remains the gold standard, it was initially used to measure the consequences of muscle lengthening by successive plaster casting [46] on muscle and tendons. Nevertheless, and to our knowledge, the only study [36] conducted after an injection of botulinum toxin in spastic muscles, did not find any decrease in passive stiffness for the triceps surae–calcaneus–talocrural joint, 6 weeks after the injection. However, in animal models, the passive elasticity module of muscle fascicles increases 1 month post-injection [25]. No potential correlation with the increased collagen and structure protein regulating the viscoelastic properties of muscles, such as the titin protein, was demonstrated. Following a more mechanical approach, the "stiffness" of muscle tissue, as a viscoelastic material, depends on the intrinsic muscle structure and its contractile status at rest. It can be analyzed via elastography imaging. The use of this non-invasive method, in real time, and without any involvement of the patient, seems adapted to the evaluation and follow-up of neuromuscular pathologies, especially spasticity and its local treatment. The reproducibility of this technique still needs to be analyzed in pathologic populations according to a well-determined procedure: experimented operator, standardized mapping of an echo-anatomical area, technical condition (control of the exerted pressure).

5. Conclusion

Even though the use of BoNtA is recommended in the literature [47–49], and its functional benefits have been acknowledged [50–52], this review of the literature demonstrates that these injections lead to structural changes in the muscle: lingering atrophy, with a remodeling of the muscle contractile proteins, which is probably not completely reversible; and changes in muscle elasticity. It seems essential, in light of the repeated injections of BoNtA in one single individual, to conduct further studies on muscular changes induced by BoNtA injections. Specifically, it requires the implementation of validated and non invasive exploration procedures, at rest and in motion. The 2D ultrasound imaging, coupled with a validated measurement procedure via elastography imaging, appears to be the best technique to meet this requirement. The challenge being to better define the indications for injections and associated treatments such as stretching, posture, lengthening casts, muscle strengthening and in some specific cases, surgical management.

Disclosure of interest

The authors declare that they have no competing interest.

References

[1] Lieber RL, Steinman S, Barash IA, Chambers H. Structural and functional changes in spastic skeletal muscle. Muscle Nerve 2004;29:615–27.
[2] Gracies J-M. Pathophysiology of spastic paresis. I: paresis and soft tissue changes. Muscle Nerve 2005;31:535–51.
[3] Huet de la Tour E, Tardieu C, Tabary JC, Tabary C. Decrease of muscle extensibility and reduction of sarcomere number in soleus muscle following a local injection of tetanus toxin. J Neurol Sci 1979;40:123–31.
[4] McLachlan EM. Atrophic effects of proximal tendon transection with and without denervation on mouse soleus muscles. Exp Neurol 1983;81:651–68.
[5] Williams PE, Goldspink G. Changes in sarcomere length and physiological properties in immobilized muscle. J Anat 1978;127:459–68.
[6] Dietz V, Sinkjaer T. Spasticity. Hand Clin Neurol 2012;109:197–211.
[7] Crosbie J, Alhusaini AAA, Dean CM, Shepherd RB. Plantarflexor muscle and spatiotemporal gait characteristics of children with hemiplegic cerebral palsy: an observational study. Dev Neurorehabil 2012;15:114–8.
[8] Simon O, Yelnik A, Bensmail D, Chaléat-Valayer E, Decq P, Dehail P, et al. Recommandations de bonne pratique. Traitement médicamenteux de la spasticité; 2009.

[9] Corry IS, Cosgrove AP, Duffy CM, McNeill S, Taylor TC, Graham HK. Botulinum toxin A compared with stretching casts in the treatment of spastic equinus: a randomised prospective trial. J Pediatr Orthop 1998;18:304–11.

[10] Flett PJ, Stern LM, Waddy H, Connell TM, Seeger JD, Gibson SK. Botulinum toxin A versus fixed cast stretching for dynamic calf tightness in cerebral palsy. J Paediatr Child Health 1999;35:71–7.

[11] Koman LA, Mooney JF, Smith BP, Goodman A, Mulvaney T. Management of spasticity in cerebral palsy with botulinum-A toxin: report of a preliminary, randomized, double-blind trial. J Pediatr Orthop 1994;14:299–303.

[12] Seyler TM, Smith BP, Marker DR, Ma J, Shen J, Smith TL, et al. Botulinum neurotoxin as a therapeutic modality in orthopaedic surgery: more than twenty years of experience. J Bone Joint Surg Am 2008;90:133–45.

[13] Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Int J Surg Lond Engl 2014.

[14] Vasilescu D, Dudea S, Botar-Jid C, Sfrângeu S, Cosma D. Sonoelastography contribution in cerebral palsy spasticity treatment assessment, preliminary report: a systematic review of the literature apropos of seven patients. Med Ultrason 2010;12:306–10.

[15] Kwon DR, Park GY, Kwon JG. The change of intrinsic stiffness in gastrocnemius after intensive rehabilitation with botulinum toxin a injection in spastic diplegic cerebral palsy. Ann Rehabil Med 2012;36:400–3.

[16] Al-Al-Shaikh M, Michel F, Parratte B, Kastler B, Vidal C, Aubry S. An MRI evaluation of changes in piriformis muscle morphology induced by botulinum toxin injections in the treatment of piriformis syndrome. Diagn Interv Imaging 2014.

[17] Choi WH, Song CW, Kim YB, Ha CS, Yang G-H, Woo H-D, et al. Skeletal muscle atrophy induced by intramuscular repeated dose of botulinum toxin type A in rats. Drug Chem Toxicol 2007;30:217–27.

[18] Dodd SL, Selsby J, Payne A, Judge A, Dott C. Botulinum neurotoxin type A causes shifts in myosin heavy chain composition in muscle. Toxicon 2005;46:196–203.

[19] Fortuna R, Vaz MA, Youssef AR, Longino D, Herzog W. Changes in contractile properties of muscles receiving repeat injections of botulinum toxin (Botox). J Biomech 2011;44:39–44.

[20] Frick CG, Richtsfeld M, Sahani ND, Kaneki M, Blobner M, Martyn JAJ. Long-term effects of botulinum toxin on neuromuscular function. Anesthesiology 2007;106:1139–46.

[21] Legerlotz K, Matthews KG, McMahon CD, Smith HK. Botulinum toxin-induced paralysis leads to slower myosin heavy chain isoform composition and reduced titin content in juvenile rat gastrocnemius muscle. Muscle Nerve 2009;39:472–9.

[22] Ma J, Elsaidi GA, Smith TL, Walker FO, Tan KH, Martin E, et al. Time course of recovery of juvenile skeletal muscle after botulinum toxin A injection: an animal model study. Am J Phys Med Rehabil 2004;83:774–80.

[23] Stone AV, Ma J, Callahan MF, Smith BP, Garrett JP, Smith TL, et al. Dose- and volume dependent-response to intramuscular injection of botulinum neurotoxin-A optimizes muscle force decrement in mice. J Orthop Res 2011;29:1764–70.

[24] Tsai F-C, Hsieh M-S, Chou C-M. Comparison between neurectomy and botulinum toxin A injection for denervated skeletal muscle. J Neurotrauma 2010;27:1509–16.

[25] Thacker BE, Tomiya A, Hulst JB, Suzuki KP, Bremner SN, Gastwirt RF, et al. Passive mechanical properties and related proteins change with botulinum neurotoxin A injection of normal skeletal muscle. J Orthop Res 2012;30:497–502.

[26] Schroeder AS, Ertl-Wagner B, Britsch S, Schröder JM, Nikolin S, Weis J, et al. Muscle biopsy substantiates long-term MRI alterations one year after a single dose of botulinum toxin injected into the lateral gastrocnemius muscle of healthy volunteers. Mov Disord 2009;24:1494–503.

[27] Dubowitz VS. Muscle biopsy: a practical approach. Philadelphia: Saunders Elsevier; 2007.

[28] Boyaci A, Tutoglu A, Boyaci N, Koca I, Calik M, Sakalar A, et al. Changes in spastic muscle stiffness after botulinum toxin A injections as part of rehabilitation therapy in patients with spastic cerebral palsy. NeuroRehabilitation 2014;35:123–9.

[29] Picelli A, Bonetti P, Fontana C, Barausse M, Dambruoso F, Gajofatto F, et al. Is spastic muscle echo intensity related to the response to botulinum toxin type A in patients with stroke?. A cohort study. Arch Phys Med Rehabil 2012;93:1253–8.

[30] Tok F, Ozçakar L, Safaz I, Alaca R. Effects of botulinum toxin-A on the muscle architecture of stroke patients: the first ultrasonographic study. J Rehabil Med 2011;43:1016–9.

[31] Raj IS, Bird SR, Shield AJ. Reliability of ultrasonographic measurement of the architecture of the vastus lateralis and gastrocnemius medialis muscles in older adults: Reliability of ultrasonography. Clin Physiol Funct Imaging 2012;32:65–70.

[32] Cho KH, Lee HJ, Lee WH. Reliability of rehabilitative ultrasound imaging for the medial gastrocnemius muscle in poststroke patients. Clin Physiol Funct Imaging 2014;34:26–31.

[33] Van Campenhout A, Verhaegen A, Pans S, Molenaers G. Botulinum toxin type A injections in the psoas muscle of children with cerebral palsy: muscle atrophy after motor end plate-targeted injections. Res Dev Disabil 2013;34:1052–8.

[34] Williams SA, Reid S, Elliott C, Shipman P, Valentine J. Muscle volume alterations in spastic muscles immediately following botulinum toxin type-A treatment in children with cerebral palsy. Dev Med Child Neurol 2013;55:813–20.

[35] Haubruck P, Mannava S, Plate JF, Callahan MF, Wiggins WF, Schmidmaier G, et al. Botulinum s. Toxins 2012;4:605–19.

[36] Alhusaini AAA, Crosbie J, Shepherd RB, Dean CM, Scheinberg A. Mechanical properties of the plantarflexor musculotendinous unit during passive dorsiflexion in children with cerebral palsy compared with typically developing children. Dev Med Child Neurol 2010;52:e101–6.

[37] Park G-Y, Kwon DR. Sonoelastographic evaluation of medial gastrocnemius muscles intrinsic stiffness after rehabilitation therapy with botulinum toxin a injection in spastic cerebral palsy. Arch Phys Med Rehabil 2012;93:2085–9.

[38] Schiaffino S, Reggiani C. Fiber types in mammalian skeletal muscles. Physiol Rev 2011;91:1447–531.

[39] Kranjc BS, Sketelj J, D'Albis A, Erzen I. Long-term changes in myosin heavy chain composition after botulinum toxin an injection into rat medial rectus muscle. Invest Ophthalmol Vis Sci 2001;42:3158–64.

[40] Legerlotz K, Smith HK, Hing WA. Variation and reliability of ultrasonographic quantification of the architecture of the medial gastrocnemius muscle in young children. Clin Physiol Funct Imaging 2010;30:198–205.

[41] Pillen S, van Keimpema M, Nievelstein RAJ, Verrips A, van Kruijsbergen-Raijmann W, Zwarts MJ. Skeletal muscle ultrasonography: visual versus quantitative evaluation. Ultrasound Med Biol 2006;32:1315–21.

[42] Kamath S, Venkatanarasimha N, Walsh MA, Hughes PM. MRI appearance of muscle denervation. Skeletal Radiol 2008;37:397–404.

[43] Hill AV. The series elastic component of muscle. Proc R Soc Lond Ser B Contain Pap Biol Character R Soc G B 1950;137:273–80.

[44] Tardieu C, Colbeau-Justin P, Bret MD, Lespargot A, Huet de la Tour E, Tardieu G. An apparatus and a method for measuring the relationship of triceps surae torques to tibio-tarsal angles in man. Eur J Appl Physiol 1976;35:11–20.

[45] Tardieu C, Colbeau-Justin P, Bret MD, Lespargot A, Tardieu G. Effects on torque angle curve of differences between the recorded tibia-calcaneal angle and the true anatomical angle. Eur J Appl Physiol 1981;46:41–6.

[46] Tardieu G, Tardieu C, Colbeau-Justin P, Lespargot A. Muscle hypoextensibility in children with cerebral palsy: II. Therapeutic implications. Arch Phys Med Rehabil 1982;63:103–7.

[47] Bertrand H, Forin V. [Botulinum toxin type A in children: evaluation of indications with a review of the literature]. Ann Readapt Med Phys Rev 2003;46:346–52.

[48] Simpson DM, Gracies J-M, Graham K, Hallett M, Miyasaki J, Naumann M, et al. Assessment: botulinum neurotoxin for the treatment of spasticity (an evidence-based review). Neurology 2009;73:736–7.

[49] Yelnik AP, Simon O, Parratte B, Gracies JM. How to clinically assess and treat muscle overactivity in spastic paresis. J Rehabil Med 2010;42:801–7.

[50] Chaléat-Valayer E, Parratte B, Colin C, Denis A, Oudin S, Bérard C, et al. A French observational study of botulinum toxin use in the management of children with cerebral palsy: BOTULOSCOPE. Eur J Paediatr Neurol 2011;15:439–48.

[51] Luauté J, Landrault E, Jacquin-Courtois S, Mertens P, Rode G, Boisson D. [Treatment of focal upper limb spasticity with botulinum toxin after stroke. Interest of an individual approach]. Ann Readapt Med Phys Rev 2004;47:555–62.

[52] Yelnik A, Colle F, Bonan I, Bradai N. [Efficacy at six months of the botulinum toxin A in the post-stroke lower limb's muscular overactivity]. Ann Readapt Med Phys Rev 2002;45:159–65.