Original Article

Correction of dynamic wrinkles with incobotulinumtoxinA

Resultados da aplicação de incobotulinumtoxinA em pacientes para correção de rugas dinâmicas

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ABSTRACT

Introduction: Patients (n = 56) received incobotulinumtoxinA for the treatment of dynamic facial wrinkles.
Objective: To test the efficacy of incobotulinumtoxinA in correcting facial wrinkles.
Methods: The toxin was applied in in the usual doses and regions.
Results: Only 38 patients returned for evaluation after treatment. Of those, 19 complained about the low efficacy, short duration or absence of effects of the toxin. The total dose applied in patients who expressed satisfaction with the outcome (19 patients) was 872 units, while in the dissatisfied group (19 patients) it was 748 units – which was later increased by an additional 30.62% (229 units) of the initial dose.
Conclusion: Although some clinical studies demonstrate that this botulinum toxin has an efficacy similar to others available, this study has not succeeded in reproducing that fact.
Keywords: botulinum toxin; face; therapeutics.

RESUMO

Introdução: *Foram selecionados 56 pacientes para receber aplicação de incobotulinumtoxinA para o tratamento de rugas dinâmicas faciais.*
Objetivo: *Testar a eficácia da toxina em questão, para essa finalidade.*
Métodos: *A toxina foi aplicada em doses e regiões usuais.*
Resultados: *Apenas 38 pacientes retornaram e foram avaliados após a aplicação, dos quais 19 queixaram-se de baixa eficácia, pouca duração ou nenhum efeito da toxina. A dose total aplicada nos pacientes que ficaram satisfeitos com os resultados (19 pacientes) foi 872 unidades, enquanto no grupo não satisfeito (19 pacientes) foi 748 unidades, posteriormente complementada com mais 30,62% da dose inicial (229 unidades).*
Conclusão: *apesar de haver alguns trabalhos clínicos demonstrando que essa toxina botulínica tem eficácia semelhante à de outras, neste estudo, esse fato não se reproduziu.*
Palavras-chave: *toxinas botulínicas; face; tratamento.*

INTRODUCTION

Many preparations of botulinum toxin type A are currently available for use, with diverse chemical denominations, such as onabotulinumtoxinA (Botox®, Allergan, Inc., Irvine, CA, USA), abobotulinumtoxinA (Dysport®, Ipsen Ltd., Berkshire, UK), and incobotulinumtoxinA (Xeomin® Merz Pharma, Frankfurt, Germany). Some studies in the literature show a similar efficacy between incobotulinumtoxin the onabotulinumtoxinA (using an equivalent dose of 1:1) and between incobotulinumtoxinA and abobotulinumtoxinA (with an equivalent dose of 1:3).[1-4] This study sought to verify the efficacy of incobotulinumtoxin in the treatment of dynamic facial wrinkles.

METHODS

Fifty-six patients (53 women and 3 men), aged 19-78, were treated with incobotulinumtoxinA (Xeomin® Merz Pharma, Frankfurt) in this short, prospective, monocentric study. According to the manufacturer, a vial contains 100 DL50 units of neurotoxin, without complex proteins, in addition to human albumin and sucrose. The study was carried out at a private practice, according to good clinical practice guidelines.

The areas of application and doses applied (in units) by region, are described in table 2. The product was stored at room temperature (15-30º C) before reconstitution and refrigerated (2-8ºC) after dilution. The product was used within 24 hours after reconstitution. The dilution of all vials was carried out by dermatologist physicians with experience in the technique, using 1.07 ml of 0.9% sodium chloride, without preservatives, in sterile vials (the excess 0.7 ml of saline solution was used to compensate the loss of liquid in the syringe).

The saline solution was injected into the vial randomly, i.e., the needle sometimes pointed towards the vial's wall and sometimes did not. All vials contained vacuum. Asepsis of the patients' skin and of the vials' rubber stoppers were carried out with 0.5% chlorhexidine digluconate solution. The patients' skin was dried with sterile gauze before the puncture was carried out. The injection sites did not receive massage or any substance after the application of the toxin. The evaluation of the toxin's efficacy was clinical and by comparing standardized pictures of each treated region, before and 15 days after the procedure. The patients were clinically re-evaluated after four months.

RESULTS

Eighteen of the 56 patients did not return for the 15-day follow-up, which prevented the collection of important data to evaluate efficacy and patient satisfaction. Of the 38 patients who returned for re-evaluation 15 days after the application, 19 (50%) did not present complaints; 19 patients (50%) reported low efficacy, short duration or absence of effects of the toxin.

Patient 1 received 15 toxin units, distributed in the glabella, forehead and periocular region, and complained about the short duration of the toxin's effect (one month).

Patients 2, 6 and 18 (Figure 1, A, B and C) presented no effects.

Patients 6 and 18 received a new application of the toxin in doses similar to the initial application, again without any effect. Patient 2 declined to repeat the treatment.

Patient 3 presented an unsatisfactory response in the forehead with an 11-unit dose. He had previously received another type of botulinum toxin (abobotulinumtoxinA) in the equivalent dose of 1:3 with a positive response and did not want to repeat the treatment in the same site. In the additional sites where it was applied, the incobotulinumtoxinA's response was satisfactory.

Patients 4, 19 and 15 received 15, 15 and 24 units, respectively, in the glabella only, and required 8, 8 and 9 units as complementary doses, respectively.

Despite having received, respectively, 20, 18, 18 and 23 units in the orbicularis muscles of the eyes, patients 5, 9, 13 and 17 needed 6, 4, 6 and 6 complementary units in those points, respectively. Patient 17 remained unaffected, and was later treated with 36 abobotulinumtoxinA units (18 units per side), in order to obtain a complete effect.

Despite having used 24 units in the glabella and 15 in the forehead, patient 7 needed complementary doses of 11 and 4 units, respectively.

Patients 8 (Figures 2 A, B and C) and 10 respectively used 16 and 20 units in the glabella, 18 and 12 units in the orbicular muscle of the eyes and 11 and 8 in the forehead. They required re-applications of 10 and 5, 8 and 4, 6 and 4 units in those regions, respectively. Patient 10 did not demonstrate a response even after the complementary dose; a third application (with an additional 4, 4 and 3 units in those areas, respectively) was necessary.

Patient 11 received 30, 20 and 15 units, in the glabella, in the periorbicular regions and in the forehead, respectively, and needed 6 complementary units in each region. As no satisfactory effect was obtained, injections of a different toxin trademark, of 8 and 4 units (in the glabella and forehead, respectively) produced a satisfactory response.

Patient 12 received 19 units in the glabella, 14 in the orbicular muscle of the eyes, 11 in the forehead and 2 in the bunny lines. He/she needed 1 additional unit in the orbicularis muscle, 5 in the forehead and 1 in the bunny lines.

Patient 14 received 10 units in the glabella and 8 in the forehead, but needed 2 additional units in the glabella and 4 in the forehead. The doses in this case were considered low, however the patient was elderly, with atrophic musculature, and had previously received similar doses of a different toxin trademark without the need for complementary doses.

Despite having received 26 initial units in the glabella, patient 16 needed 7 more complementary units. In the malar region, this patient initially received 6 units (distributed in 6 one-unit points, applied intradermally to correct superficial wrinkles) and required 1 additional unit at one of the sites.

In some cases, the complementary dose was applied on the first follow-up visit (15 days after the initial application), but rather on variable subsequent days, at the patient's request (See Table 1). Four months after the application, all patients (including those who expressed satisfaction and those who expressed dissatisfaction at the first follow-up visit) described the effect of the toxin as lasting only two months in all areas of the body where it had been applied.

STATISTICAL ANALYSIS

The data from the 38 patients who returned for the follow-up visits underwent statistical analysis. Non-parametric Mann-Whitney tests were carried out in order to evaluate whether the average doses used in the application sites in the groups with satisfactory results (S) were similar to those in the

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Table 1: Clinical characteristics, application sites and amount of incobotulinumtoxinA units applied in patients satisfied and unsatisfied with the outcomes

INITIALS	AGE	PREVIOUS TOXIN	SATISFIED DATE OF 1st APPLICATION	GLABELLA	PERIORBITAL	FOREHEAD	BUNNY LINES	NASAL SULCUS	MANDIBLE	NECK	MALAR	COMPLEMENTS
MTT	34	No	13/12/10	12	8			4 U depressor muscle of the corner of the mouth / 4 U major zygomaticus muscle				
DRSA	46	Yes	13/12/10									
LHGMD	46	No	14/12/10	24	15	18						36 days
RSS	72	No	15/12/10	16	10							
ZMME	62	Yes	16/12/10	24	12	10			12	24		
SAP	51	No	16/12/10		10							
LCM	60	No	17/12/10	26	18	15						
CCS	41	Yes	21/12/10	12	14	10						
MEBF	28	No	03/01/11		axilas							
ACSC	36	No	03/01/11	24	10	13						
PPN	32	Yes	04/01/11	15		8						
GDM	34	Yes	06/01/11	24	18	15	2					
PPLC	19	Yes	06/01/11		axilas							
FRMMF	35	Yes	10/01/11	26	18	12						
RCPS	47	Yes	11/01/11	24	15	18			12	12		
JF	28	Yes	12/01/11	8		6						
JRA	48	No	26/01/11	30	15	15	3					
MMS	63	No	14/01/10	20		6						
LJC	64	Yes	21/01/11	15	18	11						
			N =19									
			DISSATISFIED	**GLABELLA**	**PERIORBITAL**	**FOREHEAD**	**BUNNY LINES**	**NASAL SULCUS**	**MANDIBLE**	**NECK**	**MALAR**	**COMPLEMENTS**
FAB	56	?	08/12/10	4	9	2	2	4				
JGD	50	?	04/12/10	18								7 days
EPB	71	Yes	15/12/10	24	12	11			12	4		14 days
AMDM	61	?	08/12/10	15								35 days
IMH	56	Yes	30/11/10	24	20	11	2					19 days
MVGS	57	Yes	02/12/2010 +	16	24	15						51 days
FBC	42	Yes	17/12/2010	24	18	15	2					14 days
MAFG	34	Yes	15/12/10	16	18	11						35 days
RMO	51	Yes	08/12/10	16	18	3						35 days
JOC	47	Yes	02/12/10	20	12	8						33 days
CREC	49	Yes	17/12/10	30	20	15						35 days
IDV	37	Yes	17/12/10	19	14	11						32 days
SRT	48	Yes	17/12/10	16	18	6						14 days
RLDFS	78	Yes	13/12/10	10	18	8					6	14 days
PSL	38	Yes	13/12/10	24		12						21 days
FOBF	53	Yes	07/01/11	26	16	15						50 days
MCB	43	Yes	13/01/10		23						8	19 days
CGS	23	No	14/01/10									
JMMA	37	Yes	02/12/10	15								
	19 -78		15/12/10	194,0								
	anos		N =19									

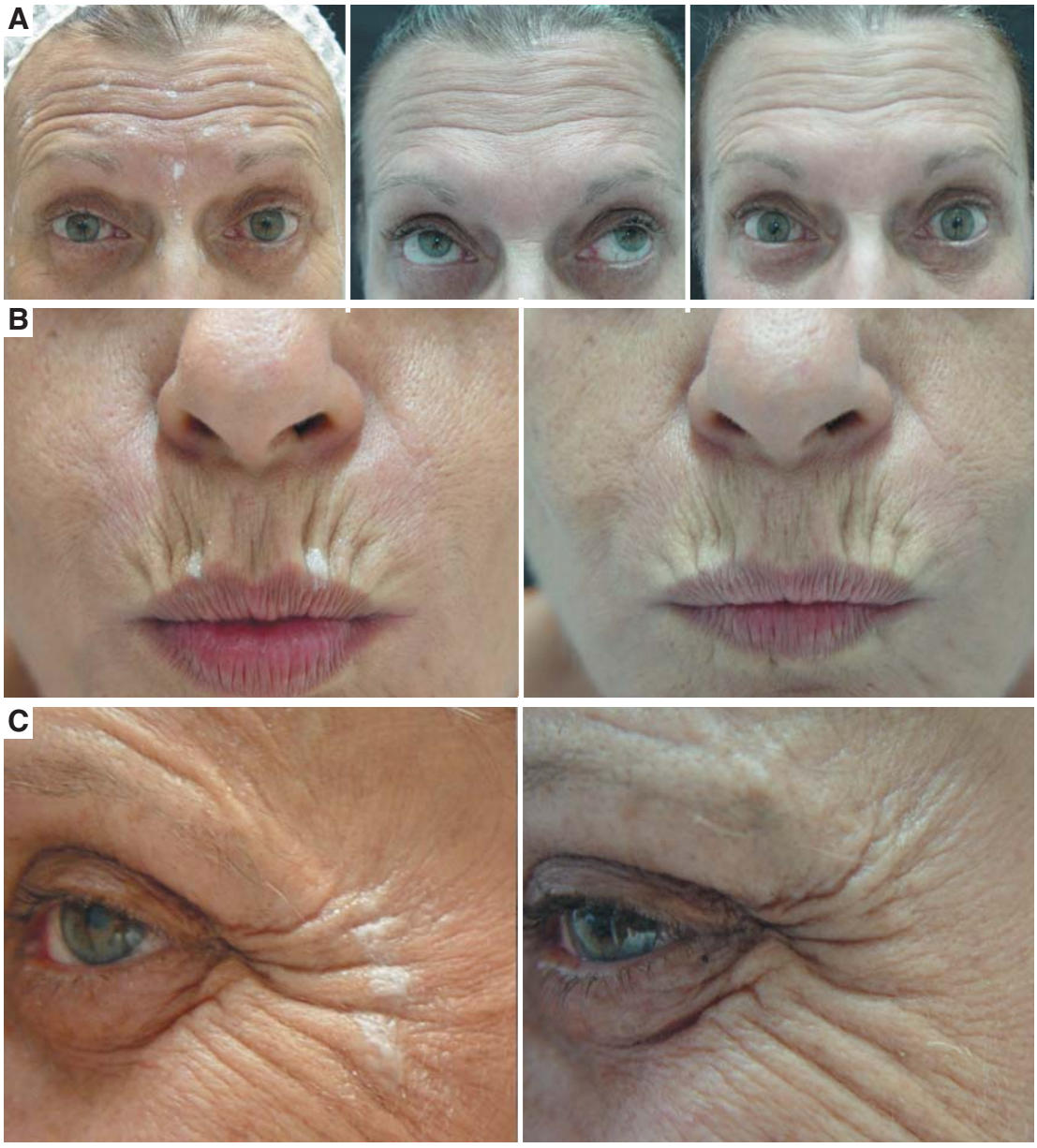


Figura 1 - A B e C.
Paciente 6, antes
e após 15 dias da primeira
aplicação e após 15 dias da
segunda aplicação

groups with unsatisfactory results (NS). It was not possible to use the parametric t-test due to the reduced size of the groups and the absence of supposition of normal distribution of the values.[5]

A hypothesis test's descriptive level of significance is the probability of obtaining more unfavorable estimates than those provided by the sample, in light of the alternative hypothesis.[6] All hypothesis tests were carried out with a 5% significance level; their respective descriptive levels (p-value) were also calculated. In this manner, only the hypotheses with p-values less than 0.05 were rejected.

The tests were conducted in order, to prove that there was no difference in the two groups regarding the variables age or doses applied. The tests were carried out using a 5% significance level, with no statistically significant differences in the average doses applied in the glabella ($p = 0.6456$), the orbicular muscle of the eyes ($p = 0.0823$) or the forehead ($p = 0.3760$) between the two groups. The test was not carried out for the remaining application sites, due to the small number of applications in both groups. There was also no difference in the patients' average age between the two groups ($p = 0.2607$). Table 2 presents some descriptive statistics for the number of units applied per dose in the first application, calculated for the area of the body and group, with satisfactory (S) and unsatisfactory (NS) outcomes.

Next, the 95% confidence interval for the proportion of patients who reported satisfaction in the follow-up visit was calculated ([34.10%; 65.90%]). The percentage values for the ratios second dose/first dose and total complementary dose (2nd + 3rd doses)/ first dose, were also computed. The latter are depicted in Graph 1.

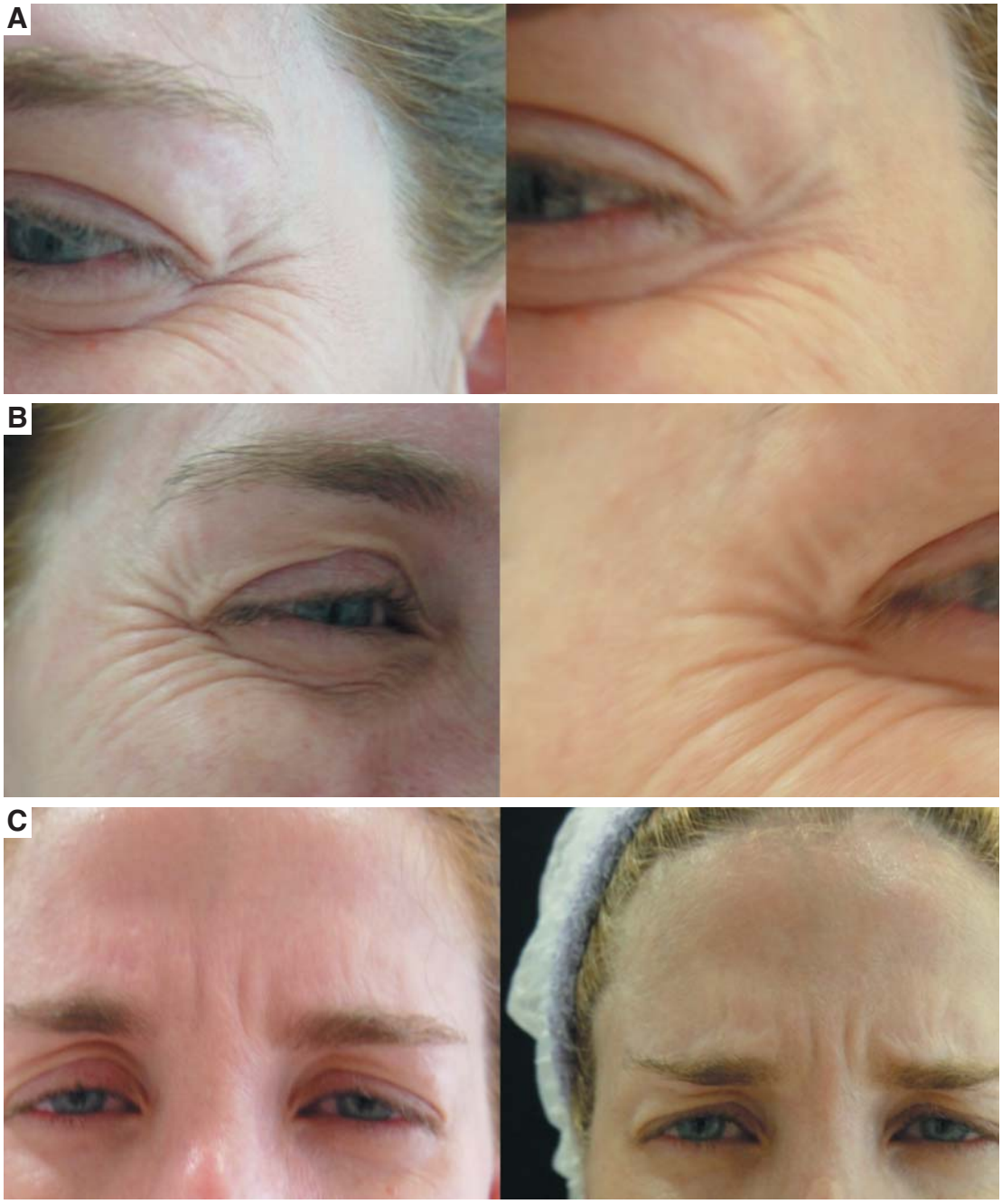


Figura 2 - A B e C.
Paciente 8, antes e após 15 dias da aplicação

Of the 19 patients who were not satisfied, 4 (21.05%) declined to receive complementary injections. A total of 30 complementary doses (2nd doses) were applied in various sites in the 15 remaining patients. The average application was 47.14% of the initial dose (minimum 7.14%, maximum 175%). The 95% confidence interval for the average percentage of the 2nd dose compared to the initial dose was [34.93%, 59.35%].

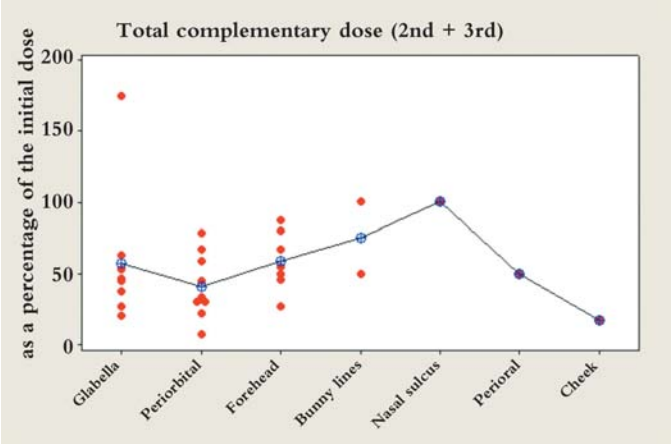
The total number of initial doses applied in the unsatisfied group was 748 units, and the total number of second doses applied in the unsatisfied group was 194 units – which corresponds to 25.94% of the first. It was also verified that the number of 3rd doses applied in the unsatisfied group was 35 units, corresponding to more than 4.68% of the value of the previous application.

Nine 3rd doses were applied in diverse sites in 3 patients only. Taking into account the complementary total dose (2nd + 3rd doses), the average application corresponded to 53.69% of the initial dose (minimum 7.14%, maximum 175%). The 95% confidence interval for the average of the percentage total complementary dose/initial dose was [41.45%, 65.93%].

Table 2: Site, satisfaction degree group, number of patients, average, minimum value, maximum value and standard error of the average for the amount of units per dose

Body site	GROUP	N.	AVERAGE	STANDARD ERROR
GLABELA	S	15	20	1,68
	NS	17	18,65	1,54
Periocular region	S	12	14,42	0,94
	NS	14	17,14	1,13
FOREHEAD	S	13	12,08	1,12
	NS	14	10,21	1,16
AGE	S	19	44,53	3,35
	NS	19	49	2,99

Satisfactory outcome (S) / Unsatisfactory outcome (NS)



Graph 1 - Total complementary doses as a percentage of the 1st dose (in red) and average percentages by application site (in blue)

DISCUSSION

Of the 56 patients treated, 38 returned to be re-evaluated after the botulinum toxin application. Of the latter, 25 had previously received previous applications of onabotulinumtoxinA (in the equivalent dose of 1:1) or abobotulinumtoxinA (in the equivalent dose of 1:3). The therapeutic efficacy was satisfactory with the previously applied toxins in those patients. Of the 19 patients dissatisfied with the outcome, 15 had earlier received one of the two other botulinum toxin type A trademarks, with the same dermatologist physicians, in the above mentioned dose proportions, with satisfactory effects and duration, and without the need for complementary doses. There are no data about the other three patients' previous applications. It is only known that one patient had never used botulinum toxin before. All patients reported the effect of the toxin as lasting only two months in all application sites.

Total or partial absence of efficacy of the incobotulinumtoxinA was verified in 50% of the patients who returned for re-evaluation. Statistical analysis demonstrated that the 95% confidence interval for the patients who were unsatisfied in the follow-up visits was [34.10%, 65.90%]. That result suggests an excessively high degree of dissatisfaction that is not usually observed in clinical daily practice with other botulinum toxin trademarks routinely used (onabotulinumtoxinA at 1:1 dose equivalence and abobotulinumtoxinA at 1:3 dose equivalence). Usually, there is no need for complementary applications. The conservation, dilution (carried out by three physician dermatologists in all cases) and application of incobotulinumtoxinA complied with the theoretical and practical instructions provided by the manufacturer at the time of the launching of the product in Brazil – when no emphasis was given to the need to point the needle towards the vial's wall when injecting the saline solution for dilution.

The manufacturer (Merz Pharma, Frankfurt, Germany) tested the batch used, confirming it was in good condition. IncobotulinumtoxinA is the most purified form of botulinum toxin, lacking complexing proteins. According to the manufacturer, complexing proteins do not seem to affect the diffusion and therapeutic efficacy of the neurotoxin, or to improve the product's stability during storage. However, they can be responsible for forming neutralizing antibodies of botulinum toxin type A 7 that can result in therapeutic failure.

One study using incobotulinumtoxinA suggests that it can remain stable for three years at room temperature – unlike onabotulinumtoxinA and abobotulinumtoxinA, which need refrigerated storage.[8] In addition, Frevert verified that incobotulinumtoxinA is the type A botulinum toxin that contains the highest specific activity of neurotoxin, when compared to onabotulinumtoxinA and abobotulinumtoxinA. This finding suggests that incobotulinumtoxinA contains active neurotoxin only, in contrast with the other trademarks, which also contain denatured or inactive neurotoxin.[9]

In animal and human models, incobotulinumtoxinA was not correlated to the development of neutralizing antibodies – even after the administration of high doses in short time intervals.[10]

In addition, despite the existence of some reports in the literature of the partial or total loss of efficacy of the botulinum toxin due to the presence of specific neutralizing antibodies, such an occurrence, which has been described with abobotulinumtoxinA and onabotulinumtoxinA, is extremely rare.[11-17] It therefore seems unlikely that all patients in this study who did not demonstrate an adequate response with incobotulinumtoxinA presented antibodies secondary to previous applications of onabotulinumtoxinA or abobotulinumtoxinA. It is assumed that high doses per application, short intervals between applications (less than 12 weeks), a high number of applications, chemical characteristics of the toxin used, and the patient's predisposition to develop an immunological reaction are risk factors for developing anti-botulinum toxin neutralizing antibodies.[18,19] Dissatisfied patients, who had received few previous applications, were treated with average doses and usual intervals. Nevertheless, the presence of neutralizing antibodies was not laboratorially evaluated.

On the other hand, it is possible that the dose proportions described in some studies (1:1 incobotulinu-

mtoxinA:onabotulinumtoxinA[20-22] and 1:3 incobotulinumtoxinA: abobotulinumtoxinA) is incorrect. Comparing incobotulinumtoxinA with onabotulinumtoxinA, Beylot verified that the former has a slightly lower efficacy in the same dose proportion.[23] In 2006, Hunt and Clarke tested three different and valid batches of incobotulinumtoxinA at a qualified laboratory, using the same trial method that Allergan uses to test the potency of onabotulinumtoxinA. That method is approved by regulatory agencies to quantify onacobotulinumtoxinA's biological activity for commercial use. Four different tests with six different incobotulinumtoxinA dilutions were carried out. The results suggested a considerably lower potency than that indicated on the packaging (i.e., 100 U/vial) compared to the potency of onabotulinumtoxinA. The average potency of the three incobotulinumtoxinA batches studied was 69-78 U/vial at the beginning of the study and 64-67 U/vial one year later. The authors proposed two explanations for this finding. The first hypothesis suggested that the different methods used by the two manufacturers (Merz Pharma and Allergan) to determine the potency affected the biological activity measurements.[24-25] The second hypothesis was that incobotulinumtoxinA loses its potency over time when stored at room temperature, perhaps due to the lack of complexing proteins that protect against the degradation of the neurotoxin, which in turn has greater exposure and reduced molecular stability.26-28 Moreover, it is also possible that both toxin trademarks have similar potencies if incobotulinumtoxinA is evaluated immediately after its manufacturing. In practice, however, the product is not used immediately after leaving the production line, but rather after variable periods of time.

A study testing incobotulinumtoxinA's efficacy in the treatment of neurological disorders (dystonia, spasticity, synkinetic reinnervation, hypersalivation and hyperhidrosis) combined with onabotulinumtoxinA in the dose proportion of 1:1 in 263 patients did not find differences regarding the appearance of the effect, duration, efficacy or side effects at doses of up to 840 U during a three-year follow-up period.4 Since the doses to treat these conditions are larger than those used in botulinum toxin cosmetic procedures, the results might be skewed in favor of incobotulinumtoxinA.

A further hypothesis that could explain the high degree of dissatisfaction verified in the present study is the requirement of special procedures in the storage, dilution and application of this new toxin. One study demonstrated that 2.5 ml and 4 ml sodium chloride solution dilutions in the preparation of the toxin for the treatment of glabellar wrinkles do not affect the aesthetic result. The patients who responded to the treatment demonstrated 100% and 89.5% improvement, respectively, two weeks after application. Three months after the procedure, those rates rose to 84.2% and 64.7%, and increased four months after to 53.3% and 61.5%, respectively. Although the study concluded that there was no apparent difference in the efficacy of the two dilutions, the data suggested a slightly higher efficacy at lower dilutions. A dilution of 1.07 ml was used in the present study, which suggests that the low efficacy was not correlated to the level of dilution.[29]

Although it does not offer an explanation for the guideline, incobotulinumtoxinA's package insert specifies that the dilution should be carried out with the needle pointing towards the vial's wall – not towards the bottom – when injecting the saline solution. Previous studies have demonstrated when diluting onabotulinumtoxinA it is not necessary to point the needle in a specific direction. Furthermore, it was shown that continuous and vigorous agitation of the vial (even to an intensity that may cause bubbles to form) and storage for up to six weeks after reconstitution do not affect the toxin's efficacy.[30-32] Therefore, it is unclear why there is advice to direct the needle towards the vial's wall. One hypothesis could be that the toxin is more fragile since it lacks complexing proteins. However, this issue not been clarified by the manufacturer.

Given these results, more studies are necessary to establish the correct equivalent doses and test the diverse storage, dilution and application conditions. Future studies should be conducted by independent investigators who have no connections to botulinum toxin manufacturers.

CONCLUSION

Although some studies demonstrate that incobotulinumtoxinA's efficacy is similar to that of other botulinum toxin trademarks, it was not possible to reproduce the duration of the effect and the efficacy of the blocking of the muscular contraction in this study. Therefore, it remains in doubt whether incobotulinumtoxinA requires any special care in its handling or whether its effects are less evident than other botulinum toxin trademarks. If so, the equivalence rate between incobotulinumtoxinA and onabotulinumtoxinA Botox® should perhaps be greater than 1:1. ●

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