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Discussion of unique properties of botulinum toxins[☆]

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1. Panel discussion

1.1. Mark Hallett

The way we are going to proceed is to go around in the same order that the speakers spoke. Each speaker is allowed to ask one question to any of the other speakers, and then we'll go around.

1.2. Mitchell Brin

Are we allowed to make comments?

1.3. Mark Hallett

Yes, you can briefly respond.

1.4. Mitchell Brin

First for the young lady downstairs and her comment about the clinical importance of what we do. We take that at heart substantially and we're investing in next generation products. This is something I will talk about this afternoon. One clarification is that we have actually different clostridial strains: the Allergan strain and the Ipsen strain are very different.

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A comment about Andy Pickett's slides, which attempted to show similarity to the Diane Tang-Liu and Roger Aoki paper (Tang-Liu et al., 2003). In the first two papers, those were saturated doses, and they were performed for different purposes and cannot really be used in the way Andy Pickett used them. The other issue is on the definitions. I think now we are all very confused, and even now Alan Scott and it sounds like Joe Arezzo, by Andy Pickett's definition, are misguided; so I'm a little concerned about that.

One of the questions I have for Andy Pickett is: in the clinical world there is a wealth of data that compare different toxins in terms of dysphagia rate, and if you look across all the toxins I think it is very clear that Dysport, Botox, and Myobloc have different profiles. Temporarily putting the animal studies aside, how do you explain that?

1.5. Andy Pickett

I would obviously dispute the data that there are differences in dysphagia between the products. I think there are very similar adverse event profiles for both products out there on the market. If you look at the data across the trials, you can pick up for example that many of the trials are in fact flawed in the way they've been carried out. When you take those away you get some decent trial data and you can show that there are very similar adverse event profiles between Dysport and Botox.

Well, if I can just make a couple of comments as well, as Mitch did. The strains of organism that we are using, the type A strains we are all using, are all different: it's very important to bear that in mind. We are all making these toxins according to different processes. So that's important

to bear in mind as well. And if you look through the literature there are hundreds of different type A complexes with different composition. That's why I'm trying to encourage my colleagues in the other companies to publish their data, so that we can all have a look at the different compositions available. But, I certainly would like to support the lady downstairs very much in this translation of animal data into clinical data. I tried very hard to show today that some of those animal data have perhaps question marks about how they were performed and how the results can be interpreted.

So I'd like to ask Mitchell Brin: you did a very nice job showing the number of patients that were treated, the number of doses of Botox that had been given, the product history, and so forth. However, I'm really puzzled as to why Allergan keeps relying on old, and perhaps disputable, animal data to talk about the safety of their product, when there's so much clinical data out there to use instead.

1.6. Mitchell Brin

Well, the clinical data clearly show the difference. I mean those are your registration clinical trials and so I think what is often done is to look at clinical data and try to sort out what's the fundamental molecular biology and what's going on. So, if we focus on clinical data, we must be reading different journals, but if we look at your registration data and our registration data, and compare the dysphagia rates, we can see they are different. This difference holds up time and time again. So I would leave it at that.

1.7. Andy Pickett

It was really the question: why does Allergan keep relying on animal safety data, or trying to interpret so-called animal safety data, at this point in time when there's so much clinical data available?

1.8. Mitchell Brin

Well, I think that there is a nice balance of clinical data and animal data. So, in our laboratories we look at different toxins trying to better understand them, and what are the key characteristics in the laboratory. At the same time, we run our clinical trials – if you look at what's in the literature – and publish that too. So, I think there is a fair bit of balance. We publish in peer-reviewed journals; it goes through the peer-review process, and we have not had reviewers' observations that fundamental animal designs are flawed or something like that. It seems to hold up to the tincture of time. But the primary focus really is on clinical data, as we've heard from many people in the audience, in terms of what holds up, in terms of the safety profile, which is absolutely critical. What is actually in the vial? That is where we end up at the end of the day treating our patients.

1.9. Joseph Arezzo

First I'd like to make a comment. One of the questions that was asked earlier was: why 200 units of BoNT/A

compared to 10,000 units of BoNT/B? This seems to be such a remarkable difference in dosing levels, but it's important to recognize that these are mouse equivalent units. The dose is based on a measure of what is lethal to mice half the time. It is not the same as for a product where the molecular weight is proportional to the dose. If dosing were based on rat equivalent units, rather than mouse equivalent units, the human dose, expressed in units, would be higher for Botox than Myobloc, because the rat is relatively insensitive to type B toxin. Obviously, the nanograms of toxin needed for a particular effect would not change, but the dose expressed in rat units would be dramatically different. So I think it's important to recognize that the 10,000 units of B does not represent that much more product as you might suspect simply by those numbers. It's the susceptibility of the mouse to the treatment. Another comment which was made during my presentation characterized BoNT/B as associated with "massive side effects." I think that statement is incorrect. There is certainly evidence for a higher incidence of some side effects with B, notably dry mouth and dysphagia, however, these reports may be more related to an increase in acceptors for B on certain types of autonomic neurons rather than greater spread from the injection site. The dysphagia is not "massive" and the complaints of this condition may be partially related to dry mouth. In the studies in which there have been long-term exposure, the incidence and severity of side effects had actually been reduced in repeated doses. I don't think it is fair to categorize the side effects of B as massive.

My questions to Mitchell Brin are two fold: 1) You showed a beautiful uniform molecular weight for BoNT/A, but what was the pH associated with those data? 2) Is the pattern similar after the product is lyophilized?

1.10. Terrence Hunt

That's the drug substance prior to lyophilisation and prior to formulation.

1.11. Joseph Arezzo

But after it's lyophilized, is it still accurate to characterize the toxin as having a uniform molecular weight, or is there free toxin present at the time of injection with a pH of 7+?

1.12. Terrence Hunt

It is not possible to run a size-exclusion on 4.4 ng of toxin; if you have an assay that can do that, please bring it to our laboratory. The reason we can only characterize the drug itself is the amount of protein in the drug product is so minuscule you really cannot assay at that point. Part of the reason is because of the excipient interferences and so forth, depending on the formulation. But we have done studies post lyophilisation and the majority of the material is complexed: the complex is maintained, is not degraded down to a pure 150 kD. I find it quite interesting that in the *in vivo* studies apparently everything is 150 kD, but in the *in vitro* studies there are differences due to molecular state. I do not understand how everything instantaneously

converts to 150 kD *in vivo*, but in the assays there are so many differences under the same conditions. It is clearly complex in our drug product (Botox).

1.13. Jürgen Frevert

At first I would like to comment to the question from the lady downstairs. I did not show the slide, but we care for patient satisfaction. And I had a slide where we compare what the patient's feeling about the treatment; we compared Botox with Xeomin and we got the same results. I've only one question, again for Mitchell Brin. I have shown data, clinical studies which demonstrate that there is no difference in the efficacy between Botox and Xeomin in two trials. Do you agree to the conclusion that there is 1 to 1 ratio concerning the efficacy of Botox compared with Xeomin?

1.14. Mitchell Brin

Looking at the design of this trial I do not think that one can actually identify the differences there, because they are underpowered to show a difference. It's a non-inferiority type of design. By my rough calculation, about 400 patients are required for each group to begin to explore that issue more scientifically. The concern I have is that there is a regulatory and legal issue, since every single regulatory agency in the world has specifically told each company that units cannot be interchanged. I will just say that the posters presented here, which make this claim, are in contrast with the advice of every agency that I visited and with the Summary of Product Characteristics (package insert). I see Andy Pickett nodding. We have a lot of experience with the agencies and making these claims will just get into trouble. It is a regulatory legal point. At this time, let the physicians use the products, understand the products; but it would be inappropriate for us to make such claims.

Andy Pickett showed these interesting slides where he took Roger Aoki's data and it looked like a double log transformation. I couldn't figure out. Terry Hunt and I were trying to figure out what was the calculation and afterwards you have to tell us. But you indicated that, when it is diluted way down, there is some breakdown in the formulation and inactivation. And that worried me a little bit. Theoretically, if there is a change, if there's additional breakdown, at a certain dosing, then the shape of the curve should change, unless the curve is specifically designed a priori not to show any difference. We do know, and some of the authors are in the room here, that with Dysport, if you add some additional albumin you get more recovery. So it is an unoptimized formulation. So your method is not clear. It looked a little bit strange, may be you should explain that to us. What's the transformation you did? How do you explain a straight-line when you say that there's a breakdown in the formulation? How did you deal with the breakdown in formulation?

1.15. Andy Pickett

Most definitely there is not a breakdown in formulation, but inactivation of toxin by excessive dilution. All the products have different quantities of albumin present in

them. When you dilute those products to clinical use, everything is fine. You recover everything that is in the vial. When you dilute down to very low LD₅₀ units, very low potency units, as I showed you in the graph, what you in fact do – and we've shown this repeatedly now for 15 years – you inactivate some of the toxin that is present, when using saline as a diluent. You have to use a protein-containing buffer to get a correct assay result. If you carry out a study where you know one product has got more albumin in it and therefore is more stabilized at very low toxin concentrations (much lower than used clinically), you are bound to get a different result (in animal studies) from one product to another. Dealing with the question on what I was doing with the data, the answer is that I was not doing anything with it other than plotting a straight-line through the points, not a curve. I was showing to you equally that the straight line, rather than a curve, is probably more applicable to the data that have been presented in those studies. Why should I draw a curve when I can use a straight line? There is also a very nice correlation coefficient of the linear data line showing parallelism of results between the products. One of the claims that has been made repeatedly in the past was that there is no parallelism between the response to the products with dose. Today I tried to show you that there is parallelism between the products, and the difference between them is simply because uniformly the doses of Dysport that have been given are lower than originally thought (by those performing the studies) because of inactivation of Dysport due to the use of saline diluent. The formulation we have is very optimal: optimal for the conditions of use in the clinic. As you remember Mitch, we had refrigeration-condition product long before you had refrigeration-condition product. We have had it ever since the beginning. It was some years before you moved out of the freezer and into the refrigerator. But I think that demonstrates that we have found a very good, stable product.

I have a question for Jürgen Frevert. I'm very interested in the adverse event data for the trials that have been published, and I think if you look quite closely at the adverse event data you will find that Xeomin, in different indications and in different trials, actually has a somewhat higher adverse event profile than Botox. Presumably all the clinicians involved in these trials are very experienced in using botulinum toxins and know how to treat the patients. So, I wonder if you can comment on this situation; I think that, if you pull the data apart, you will find a higher adverse event with Xeomin.

1.16. Jürgen Frevert

The answer is very simple. We do not find it. Well, I'm not a clinician, and I'm not involved in clinical studies, so I cannot tell anything. What I know from the statistical evaluation of all the studies is that no difference in the adverse event profile was found. Sorry, but I cannot comment on that.

1.17. Joseph Arezzo

I'd like to address my question to each of the other members of the panel. One of the most intriguing set of

observations we've had recently is the possibility of retrograde transport of BoNTs and the further observation that this transport may be serotype specific. In Antonucci's study (Antonucci et al., 2008), the two serotypes that were tested showed that there were findings consistent with the retrograde transport of BoNT/A but using the same measures, no such transport was observed for BoNT/E. If that holds to be true, do you think there would be implications for the possible differences in the transport of other serotypes, or for safety concerns, particularly in some portions of the population?

1.18. Andy Pickett

I will give a very quick answer. I think that that study is a long way from reality. Mitchell Brin and I have actually talked about this in a non-partisan way as well. I think we are both of the same opinion on this subject. Using the amount of toxin they did use, a non-validated system and all the rest, I think that the whole study is thrown into question when you try to compare it with the clinical situation. Regrettably, there was a comparison to the clinical situation embedded in that paper, and this is something which did not come up in the discussion the other day, I think that was very unfortunate. That comment, that part of the discussion, stepped outside the boundary of a scientifically interesting study.

1.19. Mitchell Brin

As Andy Pickett indicated, we've talked, Jürgen Frevert and I have also talked. Interestingly, Antonucci and colleagues (Antonucci et al., 2008) did not cite the one paper which shows that there is no evidence of transport to the brain. It is the Tang-Liu and Aoki paper that Andy put up in his presentation (Tang-Liu et al., 2003). Parenthetically, one of the flaws in using that paper for Andy Pickett's purpose is that massive doses were given to those animals, for regulatory purposes. It was a toxicology study in which 300-g rats received large doses of toxin. In the rat, very high doses (from 860 to 1147 U/kg) of IM botulinum toxin type A, as the 900 kDa complex or the free neurotoxin did not accumulate in the brain. These are obviously extremely large doses and even in this experiment, there was no evidence of botulinum toxin retrograde transport into the brain. As Andy Pickett and the whole group pointed out at this Toxins meeting, there are many flaws with Antonucci's study (Antonucci et al., 2008).

1.20. Jürgen Frevert

I totally agree. I discussed it with Mitchell Brin.

1.21. Joseph Jankovic

I have a question to all the panellists. We know that the antigenic potential of the drug depends on the protein content and we know that for Xeomin it is 0.6 ng per 100 LD₅₀ units. So the question is: what is the amount of neurotoxin in the different products?

1.22. Mitchell Brin

For Botox it is running at about 4 ng neurotoxin complex. And just to help you it is an index of mass. I think the key issue that is going on here is that you have got to go back into the clinic: that is where the answers have to come from. Clearly, we have run this very long study, a challenging study to run from the standpoint of patients being on a very prolonged clinical trial, and the data are outstanding. I think probably we are under the wire at a very low rate, at this point. And once you get under the wire, debating a little bit is not going to matter at the end of the day. I would recommend that you run the same study and take it all the way out to 15 treatments per patient, at least in some of the patients, and see where you land.

1.23. Jürgen Frevert

There is clearly an immunogenic potential in Botox because 1–2% of the patients develop antibodies and become secondary non-responder. It depends of course on the indication, but Botox induces that percentage of secondary non-responders.

1.24. Mitchell Brin

Jürgen, I would submit that you have to show that Xeomin is under that percentage. And so come back to us and let us see that study started in 2000. Come back to us with the data and show us the data!

1.25. Andy Pickett

I think it's very relevant that we have a nice mixed audience who use both Dysport and Botox (and many for a long time), who may have shown their hands earlier (when we earlier asked the audience to indicate who had seen immunoresistance). Virtually no hands were put up at all. I understand Gary Borodic's point about antibodies and he has some patients in cosmesis with immunoresistance, but I think it's a very limited number. I would agree entirely with Mitchell Brin that we need to see what the Xeomin profile looks like, sometime from now. We are in the early days and very small numbers of patients are being treated. I think we need to get other information; but, as far as we (Dysport) are concerned, this is not a significant issue.

1.26. Joseph Jankovic

I recall Andy Pickett's last slide showing that "size does not matter", but it sounds like weight does matter. So, what is the weight of each product in terms of nanograms per vial?

1.27. Mark Hallett

Doctor Frevert mentioned that there is anywhere from 4.3 to 4.5 ng per 500 units Dysport vial and that there are similar variation in other products. I just want to know what are the actual nanograms per vial. Can just each of the speakers tell us that?

1.28. *Andy Pickett*

For Dysport, as we have repeatedly published and again this month, it is 4.35 ng of toxin protein per vial.

1.29. *Joseph Jankovic*

So where did the 12.5 ng come from?

1.30. *Andy Pickett*

The 12.5 ng came from a publication by a couple of doctors in the UK well before Dysport was actually on the market: well before that. We have been back and we have looked at details and data from all of our batches, right back from the beginning of licensing and it is 4.35 ng consistently throughout. That 12.5 ng is a dreadful error that keeps getting dragged up from time to time, and is used in pretty much the way Jürgen Frevert used it, because it has some interesting advantages for other products over Dysport.

1.31. *Jürgen Frevert*

Can I comment on that? This was not an early publication! I have a company leaflet, released years after registration, and in this leaflet it said 12.5 ng per vial. And so this is an official information.

1.32. *Andy Pickett*

You will find no company literature now, at all!

1.33. *Jürgen Frevert*

Of course I agree, but at that time, and I don't know why, there was this information.

1.34. *Andy Pickett*

The information in that leaflet was based on that old publication. Then we came to realize that the publication was incorrect. Therefore all the literature on the product is now absolutely accurate and correct. Ipsen has published several times 4.35 ng of the complex, in peer-reviewed journals. So that is the figure that we have, that is the figure that we have always had. We showed batch data right the way back to the beginning. No other company has shown batch data back to the beginning of licensing as we have.

1.35. *Mark Hallett*

OK, so we have an agreement that at least the most recent data are 4.3 ng per 500 units of Dysport, 0.6 ng per 100 units of Xeomin. Mitchell can you give us the value for Botox?

1.36. *Mitchell Brin*

Approximately 4.5 ng neurotoxin complex.

1.37. *Mark Hallett*

4.5 ng! And Joe?

1.38. *Joseph Arezzo*

Myobloc/Neurobloc formulated at 5000 units per ml, contains 50 ng of protein toxin complex.

2. General discussion2.1. *Andrew Blitzer*

Thank you, I just want to make a statement. All of the companies need to be somewhat careful in trying to make conversion data. As a clinician, I've been guided by the literature and my studies, all of which were with Botox, because that is all we have available in the USA. In addition to differences in products, one also needs to look at the various muscles being injected. I have used Myobloc and I have clearly seen that there is a difference in what is recommended as a conversion factor for many of the muscles I treat. As an example, we published a paper showing a conversion factor of Myobloc to Botos at 52.3:1 (Blitzer, 2005). If you use the toxin for cosmetic indications or for hemifacial spasm, it nears about 100:1, and if you use it for cervical dystonia it can range all the way up to 150:1. So, there are major differences according to the muscles that are injected with the different products. So I think the companies should stay away from a unique conversion number and allow the literature to develop product dosing ranges for each indication.

2.2. *Joseph Arezzo*

I agree that the conversion between different toxins can be complex and often characterized by substantial differences across muscles. In fact, in the monkey, we found a range of almost 10 fold in the conversion ratio for BoNT/B and BoNT/A in small (eg. abductor pollicis brevis) and large (eg. quadriceps) muscles. These variations may be related to the degree of spindle activity in the muscle and to the relative ratio of alpha and gamma- motoneurons. There are relatively few spindles in the facial muscles the toxins and so may be less effective. I also would be very cautious in converting from the adult dose to the pediatric dose. This is an area where I think people made an overly simple conversion. They took the adult dose of BoNT/A, and multiplied it by 50 to define the starting dose of BoNT/B in children; this resulted in an overexposure in several cases. There conversion from BoNT/a to BoNT/B may differ in the pediatric population compared to adults.

2.3. *Question*

My question is about the immunogenicity again. We are under the wire but we can't use the drug more often than every 3 months. So is there a way to get under the wire and still be able to give the drug more often, or at higher doses? I think that is one of my concerns as a clinician.

2.3.1. *Mitchell Brin*

I think the issue is that we have not generated such data. We generated data based upon the regulatory request, which was to enrol patients in a study based upon clinical

practice at the time of approval, and according to the package insert. And that is the best data that we have.

2.4. Question

I would ask the other companies the same issue. If we're going to look at immunogenicity, trying to treat every 3 months, that is not optimal. So do you in studies still look at shorter intervals for injections, which would be better for the patients and would help us as clinicians?

2.4.1. Mitchell Brin

I understand and agree. I think that probably there is experiential data out there amongst all of you in treating people more frequently. In other words, we do not recommend that, but, just from talking with people, I understand that people do treat patients more frequently. Take a look at your data!

2.5. Question

I want to comment on what you have stated. It is treatment in a short interval and at higher doses? We have of course studied the immunological response.

2.5.1. Andy Pickett

Sorry, I am just a little bit confused, why do you want your patients to come back for more frequent intervals, with higher doses, when you can easily give them a nice long-lasting effect, in whatever muscular condition you choose, with less frequent intervals? I mean, it does not seem to me to be terribly logical to start to increase the frequency of injection when there is no need.

2.6. Question

I have a question to all of you on the panel especially with respect to the different data on the analytical questions that have been published or presented on posters. I would like to ask you to openly discuss if it was a good idea to have an independent lab with all the different assays, for example NIBSC (National Institute for Biological Standards and Control, UK) evaluate in a planned fashion all the different products at the same time. And if you have arguments against that please tell me what those might be.

2.6.1. Andy Pickett

I think probably the best person to answer that is Dorothea Sesardic who could say a little bit about the international study that was carried out several years ago, across all labs around the world. And we could not conclude on a standard assay or international standard.

2.6.2. Edgar Salazar-Grueso

I just want to make an observation and to give a brief statement with regards to the comments that have been made by Doctor Comella and Doctor Dressler. The immunogenicity data are important. The statement around the massive immune response is taken somewhat out of context. We are busy preparing a database which will contain nearly 800 patients in total, that has treatment

duration from 2 to 7 years, and that assessed the immune response to toxin. One of the problems that we face, with regards to immunogenicity, of course is the fact that we use an assay system that's a biological protection assay that is said to be based on neutralization, but is in fact based on a clearance mechanism of action, as we've heard over the last few days. The external validity of the so-called secondary resistance is still lacking in a major way from a clinical trial perspective. And well, clinicians, myself being a neurologist, would recognize very easily a secondary failure response in an individual patient. The validity of a quantitative versus qualitative outcome on a biological protection assay in a mouse requires us to come up with what is external validity for that assay. And determining what that is, I think, has been a challenge in this field.

2.6.3. Mitchell Brin

Can I give a very quick response? Many of us in this room participated in the clinical trial with Myobloc/Neurobloc, including myself. Those data have never been published. Allergan published their data that was generated. I think there was an obligation here to publish the Myobloc/Neurobloc data.

2.6.4. Edgar Salazar-Grueso

Mitch you're right and also you participated in the original pivotal trials as well. So there are over 10,000 samples, comprising a population of nearly 800 patients from 3 clinical trials that needed to be tested. These studies have been conducted over 12 countries. At present the data are actually being prepared as we speak, and we should see these data very shortly.

2.7. Question

This is for Doctor Arezzo. Going back to the monkey experiment, you just hint some of the possible differences between Botox and Neurobloc in their effects on distant sites, the *abductor digiti minimi* (ADM) muscle: can you comment more on that?

2.7.1. Joseph Arezzo

I'm very sorry; I missed the beginning of your question, could you please repeat it?

2.8. Question

My question is on the possible difference between Botox and Neurobloc in their effects at distant sites (the ADM muscle). You showed that Botox has effects on ADM, reduces the M wave whereas Neurobloc doesn't.

2.8.1. Joseph Arezzo

Our data indicate that when the two abductor pollicis brevis (APB) muscles in the same monkey are injected with equivalent doses of BoNT/A and BoNT/B, there is a greater reduction in the CMAP of the non-injected abductor digiti minimi (ADM) muscle in the hand treated with BoNT/A. The ADM muscle does not share a fascial boundary or a nerve with the injected APB muscle, so this finding

provides evidence for greater “spread” of the BoNT/A across the muscles of the injected hand.

2.8.2. Hans Bigalke

A final comment: One unit of product A should equal one unit of product B and any other product containing BoNT/A with respect to biological activity (not necessarily to specific biological activity).

Imagine you buy one pound of butter at Safeway's and one pound of butter at Costco's. At home you realize that the piece of Safeway's butter is much smaller than the piece of Costco's butter. Complaining about the matter at Safeway's you are told that Safeway uses its own mass standard and the masses of pieces of butter from different supermarkets are not identical. Safeway's standard is claimed to be of higher quality than any other standards used, and therefore you should buy your butter at Safeway's.

To avoid confusion of this kind, compulsory international standards were created. Obeying well-defined standards is of utmost importance in pharmacy, since underdosing would not help patients, while overdosing could harm them. The fact that only a small number of unwanted reactions are observed after injections of BoNT/A stemming from different sources may be due to its wide margin of safety. Standards have been devised for a number

of biological pharmaceuticals such as penicillin, insulin, growth hormone, erythropoietin etc. It can and should also be done for all BoNT/A containing preparations.

The present conversion rate issue has nothing to do with safety, since the margin of safety of BoNT/A is quite wide. Neither has it anything to do with science, because by definition 1 unit is equal to 1 unit. Rather, this controversy is inspired by marketing reasons and only serves to nurture confusion among doctors.

Conflict of interest

The author received honoraria from Allergan, Ipsen and Merz.

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