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Xeomin is free from complexing proteins☆

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ABSTRACT

In contrast to the other botulinum toxin products Xeomin only contains the 150 kD neurotoxin without complexing proteins which have no therapeutic function and don't influence the diffusion of the neurotoxin. In large clinical Phase III studies (blepharospasm and cervical dystonia) Xeomin showed the same efficacy and profile of adverse events as Botox. Whereas competing product must be stored refrigerated, Xeomin is stable for 3 years at room temperature.

1. Xeomin contains the lowest amount of BoNT/A with respect to units, thus, it has the highest specific potency

Xeomin is the only product on the market that contains uniquely the 150 kD botulinum neurotoxin (BoNT/A). It is not contaminated with any other clostridial protein. In the SDS-polyacrylamide gel electrophoresis of the actual drug substance the visible proteins are related to the neurotoxin itself: the heavy-chain and the light-chain; impurities are absent.

A big problem in the development of a pharmaceutical such as a botulinum toxin-based product is the formulation and manufacturing of the final drug. We have learnt from the former manufacturing process of Botox that there was a massive degradation of approximately 90% of the neurotoxin and that part of the neurotoxin was inactive and acted as a toxoid. Our task was the development of a formulation in which degradation was prevented. With the creation of Xeomin we succeeded in controlling each of the manufacturing steps and thereby preventing loss of activity during dilution, formulation and lyophilisation. With Xeomin, 100 units corresponds to approximately 0.6 ng (600 pg). Furthermore, Xeomin does not contain any inactive neurotoxin that might act as an antigen.

Let me compare Xeomin with the other competing products with regard to the amount of clostridial proteins per vial. Xeomin contains only 0.6 ng of protein, whereas in the other products the amount of protein is very much higher: about 55 ng in Myobloc, about 5 ng in Botox and the Chinese product and 12.5 ng in Dysport. If you calculate the specific activity, that means the amount of clostridial protein per LD50 unit, you have only 6 pg per unit for Xeomin and a lot more protein for the different other products like Botox with 50 pg per LD50 unit.

So what does this mean for the patient? I have roughly calculated the amount of protein that is injected into patients which are treated for torticollis (Fig. 1). This is based on a dose of 200 units of Botox or Xeomin and 600 units of Dysport. In the case of Xeomin, the patient is treated with about 1 ng of clostridial protein (which is only the neurotoxin). In contrast, the patient receives a much higher amount – approximately 10-fold higher – of clostridial protein when treated with one of the other products. Here we have no real data about the composition of the complexes so we don't know exactly how much is the amount of neurotoxin in the different products; it's only known for Xeomin. With Xeomin the patients gets the lowest amount of foreign protein. This calculation is based, of course, on the fact that 1 unit of Botox is like 1 unit of Xeomin. Is that the case?

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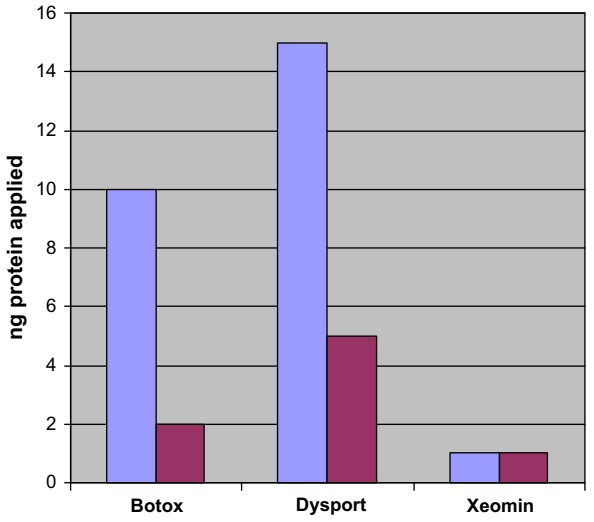

Fig. 1. Amount of clostridial protein (blue) of neurotoxin (red) applied in the treatment of cervical dystonia. Dose 200 units of Xeomin or Botox, 600 units Dysport.

2. Xeomin has the same potency and efficacy as Botox

Mitchell Brin has presented to you the results of LD50 assays of Xeomin and Botox; we were so keen to carry out equivalent experiments with five different batches of Botox and Xeomin, and we've carried it out in a blinded fashion (Dressler et al., 2008). As you can easily see in Fig. 2, the study didn't find any difference at all between the two brands. That was astonishing for us, because we use other standards. The result clearly demonstrates that there was no difference in the potency.

This was shown in the LD50 study, but how about the clinical situation? We have carried out several clinical studies to compare Botox and Xeomin. Here is the cervical dystonia trial with 460 patients – as far as I know that's the largest trial in this field – and here is the outcome: there was a reduction of 39% on the TWSTR scale in the Xeomin-treated patients and the equivalent reduction in the Botox-treated group was about 37%: both products showed the same efficacy (Benecke et al., 2005). This was confirmed for the time course of the therapeutic effect. The onset was on the same day, after about 7 days. The waning of the effects started after about 10 weeks, and the duration of the effect was about 110 days. When you evaluate the data in the Kaplan–Meyer plot you can easily see: there's no difference between Botox and Xeomin. The same is true for the blepharospasm trial, a randomised, double blind, active control, non-inferiority study with 300 patients (Roggenkämper et al., 2006). What was the outcome? After week 3, you see the same picture, there was a reduction on the Jankovic scale by 2.9 with Xeomin and by 2.7 with Botox. Again there was no difference in the time course of the therapy demonstrated also in the Kaplan–Meyer plot.

The conclusion is that the focal dystonia studies provide a consistent result that shows that Xeomin has the same efficacy as Botox, which means that 1 unit of Xeomin is equipotent to 1 unit of Botox (Jost et al., 2007).

Botox®		Xeomin®	
Lot	Potency	Lot	Potency
C1513C2	111.0	1	98.8
C1534C1	104.9	2	100.0
C1385C2	96.6	3	112.6
C1643C1	97.5	4	99.6
C1641C1	98.4	5	104.0
Mean of lots	**101.7**		**103.0**
SD	**6.2**		**5.7**
n	**5**		**5**
t-test 2-tailed			**0.734**

Fig. 2. Potency of Botox vs Xeomin. According to Dressler et al. (2008).

3. Complexing proteins play no role in the therapy with botulinum toxin but have immunostimulating properties

So let's go back to the complexing protein; that is what differentiates Xeomin from the other products. What is the function of complexing proteins? They are necessary in the protection of the neurotoxin after ingestion, and there's another speculation that the haemagglutinins presumably help in the resorption of the neurotoxin. You can draw a conclusion from that: the pure neurotoxin has a much lower oral toxicity; indeed, it was found that the neurotoxin is about 100-fold less toxic orally than the complex.

The question is why are there complexing protein in the different other products? Do they have a real function in the therapy? Here are three hypotheses about the function or the role of complexing proteins in botulinum toxin-based products. The complexing proteins might influence the diffusion of the neurotoxin. The complexing proteins can also have an effect on the stability; and, last but not least, they can influence the immune response. I will just mention shortly the hottest issue: they can potentially exert an immunostimulating effect. This is based on the fact that the haemagglutinins, which belong to the complexing proteins are lectins and lectins are known from the literature as potent in the stimulation of immune cells. In a study in 2006 by a Japanese group, with type B haemagglutinins, they showed in vaccination experiments with type B haemagglutinins and the neurotoxin – it's an experiment which has nothing to do with our clinical use – that the haemagglutinins can enhance the antibody titre against the botulinum neurotoxin (Lee et al., 2005). And they also found that some cytokines were increased in concentration. The conclusion is that there is a potential of the haemagglutinins to stimulate the immune system to produce antibodies against the neurotoxin. But this is just a possibility and there is only one study with a small number of animals which showed that Botox induced antibodies, in contrast to Xeomin, which didn't. In regard to antibody response I only can stress that Xeomin has been on the market right now for 3 years and 29,000 patients are treated, and so far we have not seen any secondary non-responders. And, as we learnt yesterday, secondary non-responders show up after 2–3 years; so we are on the right track.

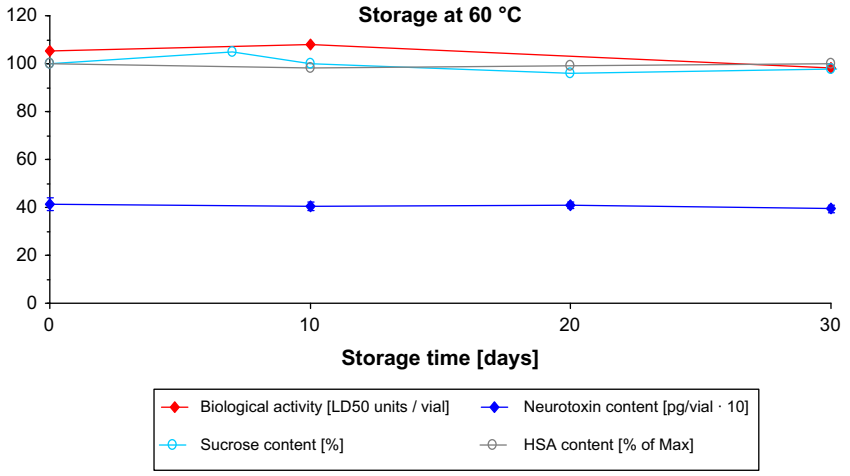

Fig. 3. Stability of Xeomin at 60 °C. According to Grein et al. (2008) Toxins, Poster #174.

Do complexing proteins influence the spread of the neurotoxin? The complex is only stable in acidic conditions. And we know on the other hand that the neurotoxin is not active at pH 6, probably because it sits inside the complex. Fortunately, under physiological conditions the complex is not stable and dissociates immediately so that the neurotoxin can act at the muscle–nerve junction. I would like to point to an interesting poster, by Eisele and Taylor (2008), which presents a study of the dissociation of the complex. They investigated the stability of the complex with ion-exchange chromatography at different pH values. At pH 6, they found a broad peak which contained the intact complex as a detailed analysis with SDS electrophoresis and protein sequencing showed. At pH 6.75, the complex was dissociating: some other proteins peaks could be detected in the chromatography representing the liberated neurotoxin and the complexing proteins. At pH 7.2 they found already 90% dissociation of the complex after 1 min. These are in vitro data which show that Xeomin – that is the conclusion – should have the same diffusion behaviour as the complex-containing products Botox and Dysport. But how is it in real life, in vivo? There are a couple of studies which investigate the spread of botulinum neurotoxin. I just want to mention two studies. There's an elegant investigation presented by Carli et al. (2008). By using immunofluorescence and Western blot technique they analysed the expression of neural cell adhesion molecule (N-CAM) in muscles adjacent to the injection site. This protein is only expressed when the muscle is denervated. They could show that there was no difference between Xeomin and the complex-containing products Botox and Dysport in the spread into adjacent muscles. Indeed, there was no spread at all of any of the products to adjacent muscles. This was also demonstrated in clinical studies, e.g. in a Phase 1B study with 32 adult volunteers applying different doses and CMAP as the parameter (Wohlfarth et al., 2007). After injection of Xeomin or Botox into the extensor digitorum brevis (EDB) muscle it was again found that Botox and Xeomin had the same efficacy. The CMAP analysis of two adjacent muscles (abductor hallucis and abductor digiti quinti) revealed that there was no reduction of the muscle activity caused by the diffusion neither in patients treated with Botox nor in patients treated with Xeomin at all doses (up to 32 units).

What do these results mean in the clinical field? If the diffusion of the product is the same then the adverse events should also be comparable. I'll show you the profile of adverse events of the two Phase III studies which I presented earlier. Again, there was no difference in the safety profile, or the adverse events profile of the different products; for example, the proportion of patients experiencing dysphagia, the most common adverse event, was 10% for Xeomin and 8% for Botox. The blepharospasm trial had the same outcome: Botox and Xeomin showed similar profiles of adverse events. So we can conclude that the diffusion or the spread behaviour of the complex-containing products and Xeomin is identical.

4. Xeomin has a high storage stability

Maybe the complexing proteins are required to stabilise the neurotoxin? We've carried out some stress experiments, where we stored Xeomin at very high temperatures. And here I show you the behaviour at 60 °C taken from Grein et al. (2008) (Fig. 3): after 30 days at this very high temperature for a protein, there was no loss of activity at all. We have shown that with the LD50 assay, and with other methods – e.g. the protease assay and in all analyses – there was no degradation detectable. And even at a higher temperature, at 70 °C, Xeomin is stable for 3 days.

To summarise a series of stability studies: Xeomin is stable at room temperature for 3 years. And we have now fresh data which demonstrates that it is stable for 4 years, at room temperature.

5. Conclusion

Let me conclude: Merz has developed a consistent manufacturing process for the neurotoxin that removes all clostridial contaminants, especially the complexing proteins.

I've shown that Xeomin has the highest specific potency. Complexing proteins, as we also know from the other presentations, do not influence the diffusion of the neurotoxin. Furthermore, the complexing proteins are not necessary for the stabilisation of the neurotoxin. Finally, Xeomin shows a similar efficacy and safety as complexing protein containing products like Botox.

I'd like to thank all my colleagues from Merz for supporting me, and thank you for your attention.

6. Discussion

6.1. Question

I understand that most of the stability data was done on the freeze-dry vial. What is the stability profile in liquid form, after reconstitution?

6.2. Jürgen Frevert

It's 24 h, like it's on the patient information and it should only be stored for 24 h at 4°, because otherwise there might be sterility problems, bacterial contamination. And to prevent that it's only for 24 h.

6.3. Question

You've always compared Botox with Xeomin, what is the stability at 60°? Have you done it too?

6.4. Jürgen Frevert

I don't know. We haven't done this experiment yet. I would like to know and to demonstrate that our product without complexing proteins is more stable at 60° compared with Botox, and we will try to do that.

6.5. M Zouhair Atassi

I think the issue of antigenicity, you have not really answered satisfactorily. You quote the paper in Microbiology, in 2006 from a Japanese group. That paper was highly flawed and I published an article subsequent to that in the same journal criticising that work. First the dose was about 1000 fold relative to what the patient gets. Secondly, the antigen immunised into the mice was actually inactivated toxin that was treated with formaldehyde that in itself causes the molecule to be more antigenic than native proteins. In fact, classically proteins that are not antigenic on their own, when you treat them with formaldehyde will become highly antigenic. Example of that in molecular immunology that cytochrome which is a small molecule is not antigenic, was thought to be not antigenic, then treated with formaldehyde, it became highly antigenic. The reason for that: the molecules are cross-linked they form polymers. And then, the next experiment used by the Japanese was cross-linking the haemagglutinin to the toxin with formaldehyde. That in itself is a flawed experiment, because in the natural complexing proteins that we see in the other products they are not treated with formaldehyde. Third, I think they used as an evidence of increased antigenicity by these haemagglutinin molecules a treatment with albumin. In the presence of these molecules with formaldehyde they showed that albumin becomes in fact more antigenic, which is expected, highly expected, because formaldehyde forms covalent cross-links among molecules of the same kind and of different kind. And so this is a very flawed study and I wouldn't quote it if I were you.

6.6. Jürgen Frevert

I apologise that I didn't mention the criticism of Dr. Atassi. I know your critique in the journal Microbiology. The only thing I wanted to say: one must be cautious, but I think nevertheless that the data allow to say that there is an immunogenic potential. A potential of the haemagglutinins to raise antibodies and this last experiment you described, there was no treatment of formaldehyde. This experiment was with HSA and isolated haemagglutinins, no formaldehyde was applied. In this experiment they have shown that the titre against HSA was increased, when they included the haemagglutinin.

6.7. M Zouhair Atassi

Formaldehyde was always used, and this is traditionally for about 50 years is known to increase antigenicity of proteins.

6.8. Jürgen Frevert

I know that, but it was not used in the last experiments.

6.9. Question

I'm making very short. Just a curiosity. You mentioned the equivalence rate between Xeomin and Botox. My curiosity is how did it come out? Was this found by chance or was the product developed in such a way to attain an equivalence? Why don't you have an equivalence with Dysport or a different potency? I mean how did this come out to be this way?

6.10. Jürgen Frevert

You mean the development of Xeomin.

6.11. Question

Xeomin comes out with this equivalence to just one product compared to others, or else an independent, say sort of potency?

6.12. Jürgen Frevert

The initial idea was: in the product you only need the neurotoxin, the pure neurotoxin. As we know now, the other proteins, the complexing proteins and all the other different proteins in other products are not necessary for the therapeutic action. And that was the idea to develop a real pure product, without any other proteins. And that is the neurotoxin. And I think we have all the same strains, and the strain produces the neurotoxin with a similar

specific activity. And you have to make sure that you don't loose this activity in the course of your manufacturing process. And so we were successful to demonstrate it.

6.13. Question

You're using the same strain as Botox.

6.14. Jürgen Frevert

Yes.

6.15. Chairman

The main issue is really why Dysport is different, I think, because you have 2 out of the 3 did the same but what is different?

6.16. Jürgen Frevert

Can I comment on that? It's a different manufacturing process. The Allergan process is directed to the 900 kD complex. And the manufacturing procedure by Ipsen is different. They have a different approach to purify and so the composition is very different. And you have seen the interesting slides by Andy Pickett. He has shown a broad spectrum of different proteins and not all the proteins are really complexing proteins There are some other proteins and they are degraded complexing proteins. So that's a totally different picture. And we didn't want to have such a problematic composition of different proteins which don't have any effect in the therapeutic treatment.

Conflict of interest

Jürgen Frevert is an employee of Merz Pharmaceuticals GmbH.

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