A Cell-Penetrating Peptide (CPP) Did Not Decrease 150-kDa BoNT/A Toxin Adsorption to Surfaces or Increase Toxin Potency or Duration in a Prototype Formulation

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CONCLUSIONS

In vitro studies indicate that addition of CPP does not prevent BoNT/A adsorption

In vivo DAS testing of prototype formulations indicate that the addition of CPP does not increase efficacy or duration of BoNT/A effect compared with BSA control formulation

These studies do not support previous claims that CPP addition offers a longer duration of BoNT/A effect

METHODS

SE-HPLC

- A CPP with the sequence RKKRRQRRRG-[K]15-GRKKRRQRRR was synthesized[3]
- A 150-kDa BoNT/A sample (Metabiologics, Madison, WI) was formulated in 50 mM potassium phosphate/150 mM sodium chloride, pH 7.2 (phosphate-buffered saline [PBS]), containing different excipients (**Table 1**)

Table 1. Excipients Tested on Size-Exclusion Adsorption Assay

Test Solution	Excipients		
	HSA (50 µg/mL)	PS20 (0.05%)	CPP (23.5 µg/mL)
PBS control	-	-	-
HSA	+	-	-
PS20	-	+	-
CPP	-	-	+
CPP + PS20	-	+	+

CPP, cell-penetrating peptide; HSA, human serum albumin; PBS, phosphate-buffered saline; PS20, polysorbate 20.

- SE-HPLC was performed in glass vials under specific different pH (6.0 or 7.2) and temperature (refrigerated [4°C] and ambient room temperature) conditions
- Samples from 3 independent studies were evaluated at various timepoints

DAS Assay

- An ≈ED_{50} dose of 150-kDa BoNT/A (≈16 U/kg) was prepared in potential formulation solutions (**Table 2**)
- Briefly, BoNT/A (150 kDa) in the formulation solutions listed was injected into the gastrocnemius muscle of the right hindlimb of mice, and animals were rated for DAS in the days following treatment. Peak DAS was captured at approximately day 2, and mice were scored for DAS until all animals in a group reached baseline for 2 consecutive days. Mean peak DAS bar graphs and duration of action curves were generated from the data (N=4–5 studies; n=6 mice per group)

Table 2. Excipients Tested on Mouse Digit Abduction Score (DAS) Assa

Test Solution	Excipients	
	CPP (0.235 µg/U[a])	PS20 (0.05%)
0.5% BSA/0.9% saline	-	-
20 mM histidine/2% trehalose buffer (pH 6.0)	+	-
20 mM histidine/2% trehalose buffer (pH 6.0)	-	+
20 mM histidine/2% trehalose buffer (pH 6.0)	+	+

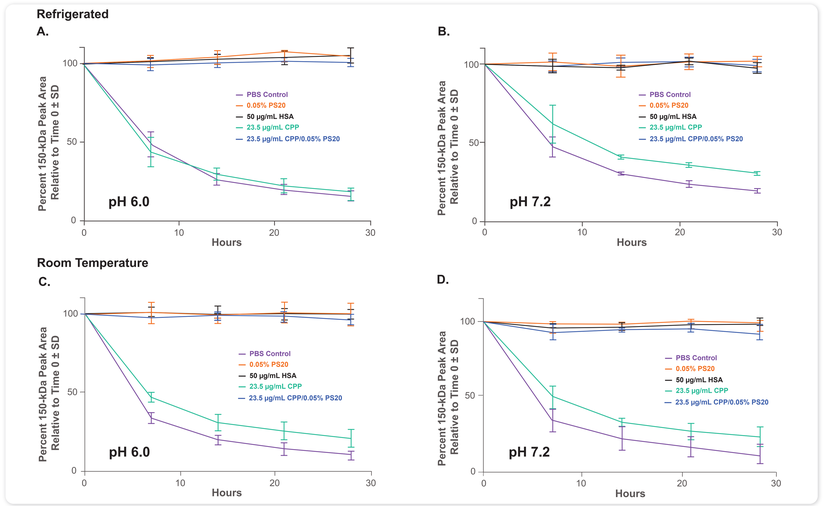
BSA, bovine serum albumin; CPP, cell-penetrating peptide; PS20, polysorbate 20.
[a]CPP/toxin ratio.

RESULTS

SE-HPLC Analysis of Toxin Adsorption

- Time course of toxin recovery (**Figure 1**)
  - Toxin adsorption to the glass vial surface was observed in PBS control and CPP-only samples at 7 hours, the first assessed time point, both with toxin recoveries of <64%
  - At 14 and 21 hours, the PBS control and CPP-only samples had decreased toxin recoveries of <42%, whereas solutions containing HSA or polysorbate 20 (PS20) continued to display toxin recoveries of >95%
- Different temperature and pH conditions (**Figure 1**)
  - CPP-only samples showed generally similar BoNT/A adsorption profiles compared with PBS controls
  - HSA and PS20 showed significantly lower BoNT/A adsorption compared with PBS controls (*P*<0.01 by analysis of variance [ANOVA]) followed by Tukey's multiple comparison test)
  - PS20 with or without CPP showed similar adsorption profiles

Figure 1. CPP Alone Does Not Prevent 150-kDa BoNT/A Toxin Adsorption to Glass Vials

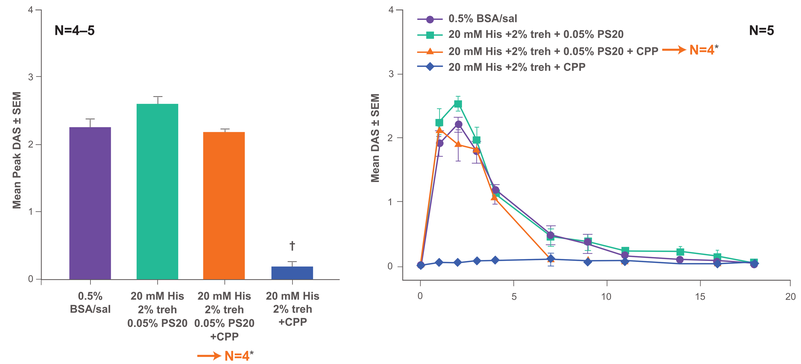


Adsorption profiles of BoNT/A (150 kDa).
CPP, cell-penetrating peptide; HSA, human serum albumin; PBS, phosphate-buffered saline; PS20, polysorbate 20.

DAS Assay

- BoNT/A prepared with CPP alone showed lower mean peak DAS compared with the formulation in BSA/saline (*P*<0.01 by ANOVA followed by Tukey's multiple comparison test) and shorter DAS duration compared with other excipients (**Figure 2**). These results suggest decreased potency and duration of effect in the presence of CPP without PS20 versus BSA
- In contrast, BoNT/A prepared in PS20 formulations with or without CPP exhibited similar mean peak DAS and duration compared with BSA/saline (**Figure 2**). These results suggest that addition of CPP does not increase DAS efficacy and duration
- Data from a rat TA-DAS assay showed a similar result, suggesting that diffusion is not responsible for the observed lower potency (data not shown)

Figure 2. CPP Alone or in Formulation Does Not Increase BoNT/A Efficacy or Duration of Action Beyond the BSA Control Formulation in the Mouse DAS Assay



(A) Mean peak Digit Abduction Score (DAS) and (B) duration of action of BoNT/A in different formulations. N=4–5 studies; n=6 mice per group. *P*<0.01 versus BSA/saline.
BSA, bovine serum albumin; CPP, cell-penetrating peptide; His, histidine; PS20, polysorbate 20; sal, saline; treh, trehalose.
*Although the mouse DAS assays were conducted in quintuplicate, there was an outlier value in one of the experiments for the assay containing 20 mM His 2% treh 0.05% PS20+CPP. These data were removed from the graph. If retained, the peak mean DAS would be 1.87 instead of 2.17.
†*P*<0.01, significantly different than BSA/saline by ANOVA followed by Tukey's post hoc analysis.

INTRODUCTION

Background

- Several botulinum neurotoxin type A (BoNT/A) formulations are currently approved or under development for a variety of therapeutic and/or facial aesthetic indications[1,2]
- Unit potencies of these drugs are not interchangeable due to unique manufacturing processes and formulations[1]
- Excipients, such as human serum albumin (HSA), are used to stabilize BoNT/A in the vial and prevent adsorption to surfaces[1]
- Previous reports have suggested that a cell-penetrating peptide (CPP) excipient stabilizes BoNT/A neurotoxin against adsorption and thermal aggregation, and may contribute to a longer duration of action
- CPP is a positively charged 35–amino acid peptide that contains a protein transduction domain at each terminus[3,4]

Objective

- The objective of this study was to evaluate the effect of CPP on BoNT/A adsorption using size-exclusion high-performance liquid chromatography (SE-HPLC), as well as toxin potency and duration of action using the in vivo mouse digit abduction score (DAS) assay

ADDL INFO

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