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Diagnosis of botulinum toxin exposure in rats

Introduction

Botulinum neurotoxins (BoNT) belong to the most toxic compounds known and are candidate compounds for use as biological weapon. In case of intoxication with these toxins adequate diagnosis is required to optimize the therapy. Sensitive analytical methodology has to be available to determine the low levels of toxins the biological matrix. In this paper we show the EndoPep assay and the ECL assay that appear to be sensitive enough to detect these toxins at relevant levels. Further the toxicokinetics of BoNT/A and B following i.v. injection, intra-tracheal and oral exposure were examined to demonstrate the usefulness of the two methods.

EndoPep Method

Using the EndoPep method, Botulinum toxins can be isolated from plasma using antibody magnetic loaded beads. Next the isolated toxin is incubated with substrate peptide which is digested by the toxin. The sequence of this peptide is inspired of the SNARE protein normally digested by the particular toxin. The concentration of breakdown products is a measure of the concentration of toxin. In this assay we used fluorescent labeled peptides and the analysis was performed with capillary electrophoresis (CE-LIF).

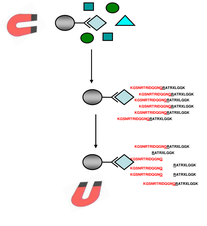


Figure 1 Outline of the sample preparation method of the EndoPep assay. Toxins were extracted from plasma using antibody loaded magnetic beads, followed by incubation with substrate peptides. .

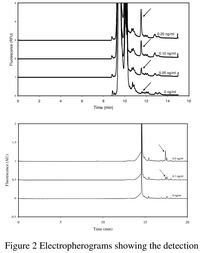


Figure 2 Electropherograms showing the detection of breakdown products of fluorescent labeled substrate peptides as result of incubation with BoNT/A (upper) and BoNT/B (lower).

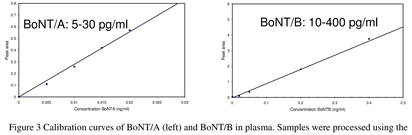
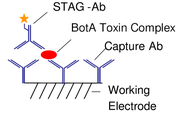


Figure 3 Calibration curves of BoNT/A (left) and BoNT/B in plasma. Samples were processed using the EndoPep assay. Plasma sample size was 100 µl. Detection limit was 1 pg/ml for BoNT/A and B.

Electrochemluminescence method

Botulinum toxin can also be sensitively detected using the electrochemluminescence (ECL) assay. The ECL makes use of the sandwich ELISA format. Detection is enabled by the Ruthenium SulfoTag labeled second antibody. If the labeled antibody is bonded to the toxin and the voltage is applied, the SulfoTag label emits light which is a measure for the amount of toxin in the sample.



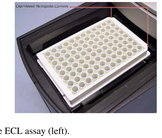


Figure 4 Detection scheme of the Sandwich ELISA using the ECL assay (left). ECL plate containing a glassy carbon electrode in each well to facilitate electrochemluminescence detection (right).

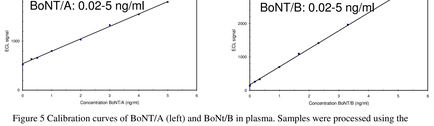


Figure 5 Calibration curves of BoNT/A (left) and BoNt/B in plasma. Samples were processed using the ECL assay. Plasma sample size was 25 µl. Detection limit was 20 pg/ml for both BoNT/A and B.

Toxicokinetics of BoNT/A and B after i.v. administration

In order to demonstrate the usefulness of the diagnostic assays, rats were i.v. injected with BoNT/A and B to study the persistence of the toxin in the circulation and distribution to the organs.

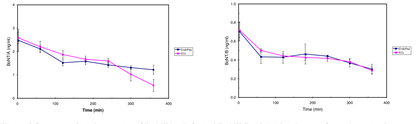


Figure 6 Concentration-time curve of BoNT/A (left) and BoNT/B (right) in plasma of rats that were i.v. injected with botulinum toxin. Rats received 40 ng BoNT/A or 10 ng BoNT/B.

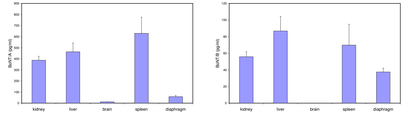


Figure 7 Concentration of BoNT/A (left) and BoNT/B (right) in tissues of rats at 8h after they were i.v. injected with the toxin.

Toxicokinetics of BoNT/A and B after intra-tracheal administration

Rats were intra-tracheally exposed to BoNT aerosols, since exposure by inhalation is one of the relevant exposure routes.

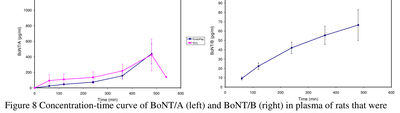


Figure 8 Concentration-time curve of BoNT/A (left) and BoNT/B (right) in plasma of rats that were intratracheally exposed to BoNT/A (left) and BoNT/B (right). Dose was 350 ng BoNT per rat.

BoNT/A could be detected in plasma of rats, already one hour after they had been exposed intra-tracheally to the toxin.

After 7 hrs the animals showed severe signs of intoxication. The levels gradually increased to an average level of 400 pg/ml within a period of 8 hrs. The animals exposed to BoNT/B didn't show any signs of intoxication and the levels of BoNT/B in plasma were much lower, 65 pg/ml. Figure 9 shows that high levels of BoNT were found in the lungs.

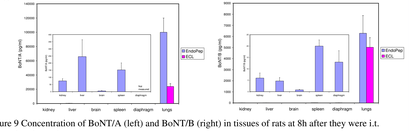


Figure 9 Concentration of BoNT/A (left) and BoNT/B (right) in tissues of rats at 8h after they were i.t. exposed to the toxin.

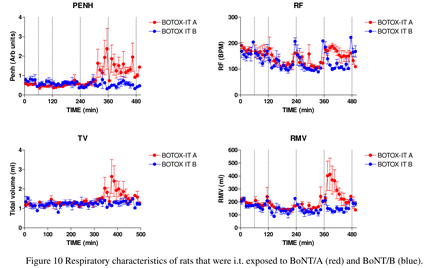
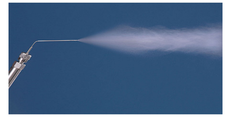


Figure 10 Respiratory characteristics of rats that were i.t. exposed to BoNT/A (red) and BoNT/B (blue). Penh = measure for bronchoconstriction, TV = tidal volume, RF = respiratory frequency, RMV = respiratory minute volume.

Figure 11 Botulinum toxin was aerosolized intra-tracheally (i.t.) using a miniature nebulizer (PennCentury, Philadelphia, PA, USA). Under brief isoflurane (4%) anesthesia, each rat was intubated with the nebulizer guided by a fibre-optic light source. Botulinum toxin was dissolved in PBS and was dispersed in the trachea just before the bifurcation.



Toxicokinetics of BoNT/A after oral administration

Ingestion is another important exposure route in case of botulinum toxin intoxication, for example food poisoning. Botulinum toxin (10 µg) dissolved in drinking water was administered orally by gavage (OD 1.5 mm) in a volume of 1 ml/kg. However, the toxin was not detected in plasma when botulinum toxin in the purified form was administered. The animals didn't show any symptoms either.

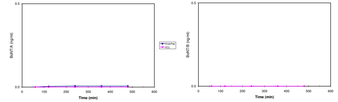


Figure 12 Concentration-time curve of BoNT/A (left) and BoNT/A complex (right) in plasma of rats that received BoNT/A pure toxin or complex orally by gavage.

Conclusions

- Analytical methodologies for detection of botulinum toxin A and B were developed using EndoPep and ECL.
- EndoPep: LOD 1 pg/ml for both toxins (100 µl sample)
- ECL: LOD 20 pg/ml both toxins (25 µl sample)
- Slow elimination of BoNT/A and B after i.v. administration
- No toxin detected after administration in the stomach by gavage
- Intra tracheal aerosol exposure leads within 1h to detectable levels of BoNT/A and B in blood

References

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