Development and validation of an in vitro assay for the determination of tetanus toxicity

Project team

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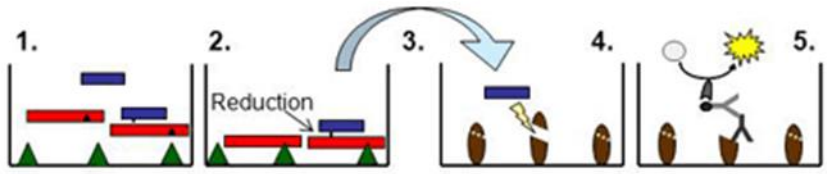
Project summary

Tetanus vaccines are produced from tetanus neurotoxin (TeNT) which has been detoxified by formaldehyde treatment. According to the European Pharmacopoeia, the resulting toxoid bulks have to be tested for "absence of toxin and irreversibility of toxoid" by injecting them into guinea pigs. If none of the animals dies or shows tetanus symptoms after the injection, the toxoid is regarded as safe. Our aim is to provide a reliable in vitro method for the function-based detection of active TeNT which is able to replace these animal tests.

On the molecular level, TeNT consists of two subunits: The heavy chain mediates the binding to neurons and the subsequent uptake into these cells, while the light chain acts as a protease which specifically cleaves the protein synaptobrevin inside the neurons. This cleavage induces a spastic paralysis which is characteristic of tetanus infections.

In order to closely mimic a tetanus infection in an animal-free system, our project group has developed a "Binding and Cleavage" (BINACLE) assay. This assay detects active TeNT molecules based on their specific receptor-binding and proteolytic characteristics: Only toxin molecules which possess a functional binding domain as well as an active enzymatic domain will generate a signal in this assay. Thus, the BINACLE assay can more reliably discriminate between toxic and inactivated TeNT molecules (and therefore is less prone to false-positive results) than previously described in vitro assays which are based on measuring only the proteolytic activity alone.

Further studies are now being performed to examine the applicability of the method for the safety testing of tetanus vaccines. In-house validation results have demonstrated that the sensitivity of the BINACLE assay is equivalent to the sensitivity of the animal test, and that the assay is able to detect TeNT which has been artificially spiked into toxoid samples from relevant vaccine manufacturers. The lab-to-lab transferability of the method has been shown in an international transfer study. In order to further validate the assay and to promote its acceptance and usage as an alternative safety test for the quality control of tetanus vaccines, an international collaborative study is currently being performed in collaboration with the European Directorate for the Quality of Medicines and HealthCare (EDQM).



Principle of the combined test system: (1.) Tetanus toxin molecules bind via their heavy chains (red) to immobilized receptor molecules (green) on a microtiter plate. (2.) The addition of a reducing agent leads to the release and activation of the light toxin chains (blue). (3.) The light chains are transferred to a second plate which has been coated with the substrate protein synaptobrevin (brown). (4.) The light toxin chains specifically cleave the synaptobrevin. (5.) The cleavage fragment is detected by means of an antibody, resulting in a color signal which is measured photometrically.

Source: PEI

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Awards

- In 2010, the project team was awarded an animal welfare research prize (29. Forschungspreis zur Förderung methodischer Arbeiten mit dem Ziel der Einschränkung und des Ersatzes von Tierversuchen) by the German Federal Ministry of Food, Agriculture and Consumer Protection.
- In 2016, Heike Behrensdorf-Nicol was awarded the ALTEX Prize by the Doerenkamp-Zbinden Foundation as first author of a review publication describing the BINACLE assay for detection of tetanus neurotoxin [ALTEX 32:41-46, 2015].