Stiftung zur Förderung der Erforschung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen

Project

Development of an *in vitro* method for the determination of tetanus toxicity in tetanus vaccines

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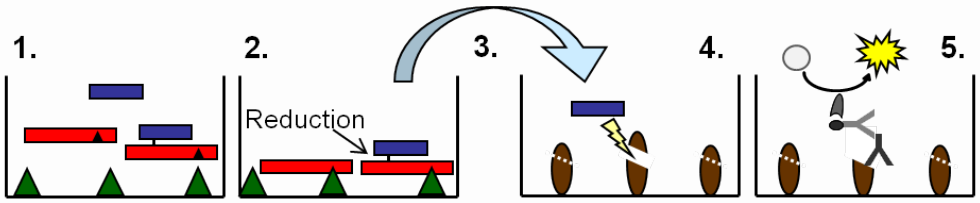
Tetanus vaccines are made from the neurotoxin of the bacterium *Clostridium tetani* which has been chemically inactivated. Following the inactivation procedure, the resulting tetanus toxoid has to be tested for the "absence of toxin and irreversibility of the toxoid". According to the respective monographs of the European Pharmacopoeia, these toxicity tests have to be performed in guinea pigs: samples of each toxoid bulk must be injected into a total of 15 (for the production of human vaccines) or 10 (for veterinary vaccines) animals, which are then monitored for tetanus symptoms. The aim of our project is to develop an *in vitro* method for the determination of active tetanus neurotoxin (TeNT) which can replace these animal tests.

As cell culture-based models for the detection of tetanus toxicity do not offer an adequate level of sensitivity, our project focuses on the functional detection of TeNT by means of biochemical methods. Each TeNT molecule consists of two subunits: The heavy chain mediates the binding to neurons and the subsequent uptake of the toxin into the cells, whereas the light chain contains a protease domain which specifically cleaves the neuronal protein synaptobrevin. This cleavage blocks the release of inhibitory neurotransmitters and thus induces the severe muscle spasms which are the characteristic symptoms of a tetanus infection.

In former studies, our project group had shown that methods which only measure either the toxin's protease activity or its ability to bind to neuronal receptors do not allow a reliable determination of tetanus toxicity. Such methods which are based on only one functional parameter are highly susceptible to false-positive results caused by free toxin subunits.

We have therefore developed a combined test system which takes into account the functional integrity of both TeNT subunits. In this combined test system, TeNT molecules can only generate a signal if they possess a functional binding domain as well as an active protease domain – and if both domains are present on distinct subunits which can be separated by reduction.



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.

We have demonstrated that this combined test system is able to distinguish between toxic TeNT and non-toxic tetanus toxoids. In addition, the method allows the detection of TeNT in toxoid samples which have been artificially spiked with active toxin. Hence, the combined test system basically allows the determination of tetanus toxicity on a functional basis. Before the method can be used as an alternative to the toxicity tests in guinea pigs, however, particularly its sensitivity has to be further improved. The main goal of the experimental work in the funding period is therefore the optimization of various assay parameters in order to reach a detection limit which is equivalent to the animal test. In addition, it has to be determined whether the *in vitro* test system can be applied to toxoid batches from all relevant vaccine manufacturers. The ultimate goal of the project will be to replace the mandatory *in vivo* toxicity tests for tetanus vaccines by the combined *in vitro* test system.

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