Lipid vesicles to replace mouse experiments

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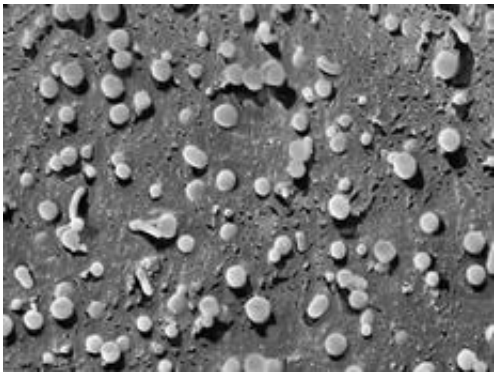

Liposomes modelled according to nerve endings could replace mice as a test system for determining Botox activity in future. Credit: Electron Microscopy ETH Zurich - EMEZ

Researchers from ETH Zurich have filed a patent application for a method to test the biological activity of one of the strongest toxins known, the botulinum neurotoxin. If the procedure is adopted by the pharmaceutical industry, it could save the lives of more than half a million mice per year.

Produced by the bacterium *Clostridium botulinum*, the Botulinum Neurotoxin (BoNT) is one of the strongest poisons known to man. BoNT inhibits the signal transmission from nerve endings to the muscles, causing anything from paralysis to apnoea. One microgram per kilogram of body weight is sufficient to kill an adult human.

BoNT is a notorious foodborne poison, sometimes found in insufficiently sterilised canned vegetables. However, the microbes can also thrive in meats and sausages and produce the toxin. The bacterial spores are practically everywhere but can only germinate and produce the toxin if the environment is low in oxygen and not acidic.

Over 100 different medical applications

Nevertheless, the botulinum neurotoxin also has its advantages: since the 1980s, it has been used to treat dozens of chronic conditions and deficiencies, including nerve-related malpositions such as wry neck, excessive sweating, strabism, migraines, and even the tennis elbow. And since the 1990s, the cosmetics industry has been using the once feared 'sausage poison', the brand name 'Botox', on a grand scale to smooth out face wrinkles.

Using BoNT, however, is sort of playing with fire. The toxin is a natural product and produced by bacteria, albeit not in a constant concentration and activity. Consequently, the regulatory authorities require the toxicity of every batch of a therapeutic agent containing BoNT to be tested with the mouse LD50 test, which determines the dosage at which half of the animals die. For such routine tests in the pharmaceutical industry of the EU and the USA, over half a million mice a year have to lose their lives.

Animal-free test system devised

A new test system developed by ETH-Zurich researcher Oliver Weingart in collaboration with the Spiez Laboratory could now save these mice's lives. The system is the first to work without laboratory animals or living cells, as it measures the toxic activity of the neurotoxin with the aid of artificially produced lipid membrane vesicles (so-called liposomes).

At the Laboratory of Food Microbiology, the researchers developed reaction compartments surrounded by a lipid double membrane, which mimics the ends of nerve cells. Embedded in the lipid membrane are specific nerve-cell receptors, to which the botulinum neurotoxin can bind, based on a lock-and-key principle. Through the acidification of the surrounding medium, the structure of the toxin changes so that part of it is channelled into the liposome. There, the toxin becomes active and cleaves a reporter protein present in the bubble that begins to glow after cleavage. This fluorescence is directly linked to the BoNT concentration: the brighter the liposomes, the higher the concentration of the toxin.

An inexpensive and easy-to-use assay

The proposed test system has obvious benefits. "The liposomes are easy and inexpensive to produce, and no special training is needed for handling by laboratory staff," says Weingart. And the novel system also has another advantage, since it can be modified in order to test biological activity of other toxins composed, like BoNT, of different sub-units, including tetanus, diphtheria, Shiga. or cholera toxins.

"The system is very sensitive," Weingart continues. Employing mice, the detection limit for botulinum neurotoxin is about ten picograms. The aim is to be able to detect less than one picogram of the toxin – a billionth of a gram – with the aid of the liposome assay. The system already yields a first signal in one to three hours, and the final result is available in less than twenty-four hours; whereas with mice, it can take up to four days.

It has been demonstrated that the system is capable of detecting BoNT. "We have provided a proof of concept for our initial idea" says Martin Loessner, professor of food microbiology. The researchers have recently started to standardise the test and to further improve the liposomes in order to facilitate reliable measurements.

Adaptable set-up of the test

A research team from Hannover Medical School, the company miprolab GmbH based in Göttingen, and the Spiez Laboratory, where Weingart started the project as a PhD student before joining Professor Loessner's group as a post-doc, are also involved in the development of the new test system. Together with the Federal Office for Civil Protection, with which the Spiez Laboratory is affiliated, the researchers have now filed a patent to secure intellectual property for their new test system.

Thus far, the focus has been on the needs of the pharmaceutical industry. However, Martin Loessner and Oliver Weingart also envisage expanding the test system for other applications. For example, it would be conceivable to develop a similar test for drinking water or food, to trace neurotoxins.

Currently, the project is primarily funded by a grant to Weingart from the "3R Research Foundation", which is committed towards minimising, avoiding and improving animal testing and maintained by animal protection and the pharmaceutical industry. As the foundation chooses projects very selectively, Weingart rightfully considers his fellowship as a significant honour.

Provided by ETH Zurich