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Synthetic antivenoms for snakebites: a slithering road!

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Since Adam and Eve, humankind has been equally frightened and fascinated by snakes. The ancient Egyptians worshipped the cobra, which decorate sarcophagi and the famous mask of Tutankhamun. Even today, the snake is integral to the Staff of Asclepius, a symbol of medicine. To some extent, medicine has forgotten about the snake, however, bites from venomous snakes still pose a deadly impact on people in low-and middle-income countries. It is estimated that venomous snakes kill between 81,000 and 137,000 people every year, and maim around 400,000, which represents a huge impact both on families and social economics. Around 125 years ago, Calmette began to produce antivenom serum for snakebite victims. Since then, production methods have not changed significantly; then and now, antivenom is made by immunizing animals. Isolated antibodies can neutralize some of the most lethal toxins, thereby saving many lives. However, there is still a need for animals such as horses or sheep, as well as a snake farm, in order to acquire enough venom for immunization procedures. Animal-derived antibodies pose a threat, because they are foreign components injected into the human body, which can give rise to adverse immunogenic reactions. In a worst-case scenario this can lead to anaphylactic shock and death. Synthetic antivenom, is an interesting avenue, which could reduce or entirely remove the need for immunized animals and snake farms. Synthetic antivenoms could be made to high purity and eliminate many current challenges, such as batch-to-batch variations, high costs, limited shelf life and the need for 'cold-chain' transportation.

Is there a problem with venomous snakes?

Venomous snakes are unlikely to be a problem if your home is in Europe. However, if you live in South America, Africa or South East Asia, then they are more likely to be a reality for you, your family or someone you know. In many developing countries, people work in the fields, plantations, forests or similar. Such habitats are also hunting grounds for venomous snakes, often seeking out rodents. Alongside this, primitive housing, litter (which attracts vermin) and the continuous expansion of villages and cities, increases the risk of encountering snakes. In the ocean, venomous sea snakes present a threat to those in the water, and especially to fishermen who accidently get bitten whilst working (which might actually involve hunting these very snakes, since they are considered a delicacy in some parts of the world). In 2017, the World Health Organization (WHO) placed snakebite on its list of the world's most neglected diseases. It is estimated that venomous snakes kill between 81,000 and 137,000 people every year, and maim around 400,000, leaving a huge impact on family life and their economic opportunities. Compared with other 'traditional' neglected tropical diseases, such as cholera (69,000 deaths per year), leishmaniasis (24,000) and Chaga's disease (8000), snake envenomization is therefore the largest killer.

Can't we just give antibodies to all snakebite victims?

As noted earlier, the methods for producing antivenom have changed little during the past century. Then and now, antivenom is made by immunizing animals and isolating the antibodies that can neutralize some of the most lethal toxins, thereby saving many lives. Many of these antivenoms are very effective, but these are often

expensive, and far beyond the reach of a fieldworker from Africa. Other antivenoms do not work as well, either because they are inherently poor quality or because they were developed as treatment for venom of a different species of snake to the one that has bitten the victim. Some antivenoms can cost up to $2000–3000 per vial, and treatment often requires 5–6 vials. Even if the cost is lowered and we reach a treatment with antivenom around $200–300, this is not affordable by most people in developing countries.

Making antivenom is a complicated and lengthy procedure. First, you need to have a large number of venomous snakes of the species against which you want to raise an antivenom. You then have to milk the snakes, but this often gives only a low yield. Even a relatively large sea snake, for example, often produces no more than a 150 µl. A large puff adder delivers more, around 600 µl. You then have to inject it into an animal, e.g., a horse, and wait; it often takes around a year before the horse has enough antibody to be bled for the antibody of interest. Thereafter several purification steps are necessary. Even then the desired neutralizing antibody is only a small part of the entire antivenom. Consequently, antivenoms can consist of many impurities, which can lead to adverse reactions. Furthermore, the more impurities, the more vials you need to neutralize the target venom, which also increases the risks of anaphylactic shock.

If the pharmaceutical industry could make better quality antibodies or a synthetic version, then perhaps we would have cheaper, safer and more efficient antivenoms. However, one of the major problems right now is not the lack of new and better (e.g., synthetic) antivenom, but rather lack of access to the existing ones. In a study carried out in 2010, it was estimated that only 2% of people bitten by venomous snakes in sub-Saharan Africa have access to quality antivenom treatment.

Some of the antivenoms used today are quite cost-effective. Unfortunately, we just cannot get enough to

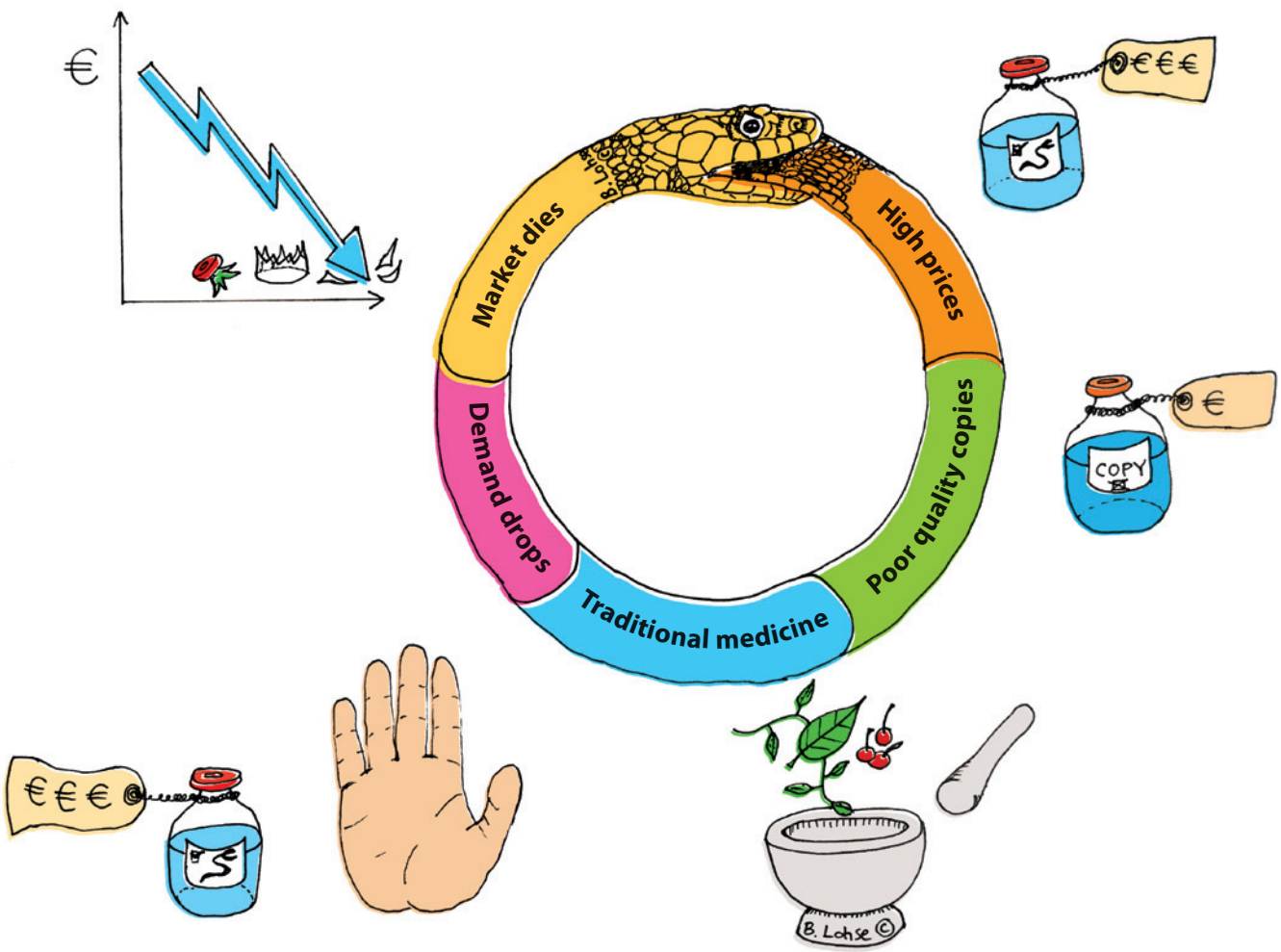

Figure 1. The ouroboros of antivenom shortage, showing that even though we have good-quality antivenom, it is outcompeted by the poor-quality copies, which do not work properly or even cause illness. People then go back to traditional medicine, and seek other aids, leading the demands for the good (but expensive) antivenom to drop. This eventually leads to companies discontinuing production and the market dies, a vicious circle as Médecins Sans Frontières termed it.

accommodate all the snakebite victims. This is due to the discontinuation of production; an example of this is Sanofi Pasteur's discontinuation of Fav-Afrique® a polyvalent antivenom previously used to treat envenoming by some snake species of sub-Saharan Africa. Today, we face a situation termed the antivenom crisis (see Figure 1, 'the ouroboros of antivenom shortage' illustration).

Why doesn't the pharmaceutical industry solve the antivenom crisis?

The vicious slithering road to antivenom shortage is a cause and effect scenario, leading ultimately to pharmaceutical companies avoiding the market altogether. This is not a cheap venture. As mentioned earlier, snake farms and horses in which to raise antibodies are a necessity. Costs include training staff to take care of the animals, milk the snakes on a regular basis, plus vet bills. Production, purification and shipping costs drive up the price. From a business point of view, low market demand, which in turn prohibits a good economy scale for antivenom manufacturers, is a major disincentive. Furthermore, the poor quality of some products, that either do direct harm or do not work, creates mistrust in the market and people turn to the local medicine man or healer instead. For many pharmaceutical companies, antivenom manufacture is simply not a worthwhile venture. The few quality products (such as CroFab®), therefore, demand high prices, which are further inflated by the insurance companies. At the extreme end of the scale, snakebite treatment at a US hospital, might cost $100,000! Fortunately, most treatments are much cheaper. However, even if a quality antivenom vial can be acquired for $300, or less (e.g., Echitab-Plus-ICP) that is still roughly 6 months of salary for a Swazi farmer! Newer brands of antivenom sell for $30 or less per vial, however, most often the quality is poor, it does not work or in worst cases it can kill you.

So how can we solve the antivenom crisis?

The WHO are working on a roadmap to tackle snakebites. Médecins Sans Frontières (MSF) is perhaps the most important player, as they give snakebite victims antivenom free of charge at their clinics around the world. In Africa many lives are saved every day because MSF can administer good-quality antivenom to snakebite victims, in for example, Abdurafi in Ethiopia. MSF also collaborates with researchers and have many good initiatives to increase awareness and knowledge of snakebites. In their access campaign, MSF has described the steps necessary for achieving a successful long-term plan (www.msfaccess.org). These include:

- A list of safe and effective antivenom products.
- These quality antivenoms should be available free of charge or at a price all can afford.
- Hotspots for snakebites in each country need to be mapped, to choose the correct antivenom.
- Quality antivenoms must be stockpiled, nationally and regionally.
- R&D must be better financed in order to improve existing antivenoms and facilitate the next generation of antivenoms.

The next generation of antivenoms

The next generation of antivenoms could be synthetic, made in the lab using DNA recombinant technology, peptide synthesis and/or chemistry. The three main research fields are currently: 1) antibody-based, 2) peptide-based and 3) small-molecule antivenoms. There are also other strategies and initiatives, such as nanoparticles and large synthetic macromolecules.

The vast majority of work continues to involve various strategies for manufacturing antibody-based antivenoms. Major breakthroughs have made it possible to undertake antibody engineering, in order to make improved versions of existing antibodies. In 1985, George Smith developed an elegant method known as phage display, where a bacteriophage—a virus that infects bacteria—can be used to evolve and identify new proteins (antibodies) or peptides, which can be used as ligands for developing new synthetic antivenom. Gregory Winter used phage display for the directed evolution of antibodies, with the aim of producing new pharmaceuticals. The first one based on this method, Adalimumab, was approved in 2002 and is used for rheumatoid arthritis, psoriasis and inflammatory bowel diseases. Since then, phage display has produced antibodies that can neutralize toxins, counteract autoimmune diseases and cure metastatic cancer. In our research at UCPH, AntiVenom Venture (AVV) focus on identifying and developing new antivenoms based on peptides. We use phage display to attempt to find new peptides that can bind and neutralize snake toxins. We also seek naturally occurring peptides and proteins, where part of these can be used as scaffolds to neutralize snake toxins. I have designated this class of compounds Serpentides™. These are peptides or peptide-based compounds that can bind and/or inhibit snake toxins. They can be naturally occurring compounds, or part of a larger protein, where this interacting part is synthesized individually, or a peptide identified through, e.g., phage display. I find this

an interesting and different strategy, from the antibody-based strategy. These Serpentides could be a new and interesting alternative to the antibody-based strategy that most are pursuing. There are many advantages with a synthetic peptide-based antivenom, such as ease of chemical modification, smaller molecular weight, higher shelf stability, larger formulation possibilities and knowledge base for designed PD and PK. However, there are also disadvantages, such as short half-life in blood and solubility issues, but these could potentially be improved via chemical modifications. Crystallization of the peptide complexed with the target is often possible, providing important knowledge about peptide–toxin interactions.

Additionally, peptides can penetrate cells and skin, thereby giving new possibilities for treatment delivery. Serpentides could be formulated as an inhalant, as skin cream (for topical application at the location of the bite) or subcutaneous, in combination with, e.g., traditional antivenom.

As it stands, we currently remain a long way from clinical application of peptides as antivenom. However, this is exactly the kind of strategy needed to expand existing routes to antivenom production. A push for new treatments for snakebites could literally prove life-saving for some of the most vulnerable people on the planet. ■

Brian Lohse is the founder and CSO of EpiDiscoverY, a CRO performing phage display technology to identify future lead structures for pharmaceuticals and fluorescence-based assay development for companies and collaborations with academia. He is also leading the AntiVenom Venture, a consortium developing tool compounds and inhibitors against snake toxins. The newest initiative from Brian, the Serpentides, is a welcome addition to antivenom research. Brian is collaborating with Médecins Sans Frontières and was recently in Ethiopia to visit the Abdurafi clinic, with regards to snakebite envenoming. He has been an Associate Professor at the University of Copenhagen since 2012. Email: bril@sund.ku.dk

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