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Biological toxins of potential bioterrorism risk: Current status of detection and identification technology

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ABSTRACT

Biological toxins are a heterogeneous group of compounds that share commonalities both with biological and chemical agents. Based on their availability, toxicity, and the lack of medical countermeasures as well as their known history of military research, toxins such as ricin, botulinum neurotoxins, staphylococcal enterotoxins, and saxitoxin are classified as toxins of bioterrorism risk. At the same time, they are known to cause naturally occurring intoxication. Different technologies for toxin detection have been established, but hardly any universally agreed reference methods or reference materials are available. Regular proficiency tests have been lacking for most of the mentioned toxins. Therefore, objective comparison of method performance has not been possible. The recently completed EU-funded project EQuATox delineated the current *status quo* of toxin detection on the basis of a series of proficiency tests. This review provides an overview of the results obtained and highlights the need for future developments in the field.

Contents

Abbreviations: AOAC, AOAC International; BoNT, Botulinum neurotoxin; CRM, Certified reference material; CWC, Chemical Weapons Convention; EFSA, European Food Safety Authority; ELISA, Enzyme-linked immunosorbent assay; EQuATox, Establishment of Quality Assurances for the Detection of Biological Toxins of Potential Bioterrorism Risk (EU project); ESI, Electrospray ionization; ESM, European screening method; HC, Heavy chain; i.p, Intraperitoneally; IUPAC, International Union of Pure and Applied Chemistry; LC, Light chain; LC-FLD, Liquid chromatography-fluorescence detection; LFA, Lateral flow assay; mAb, Monoclonal antibody; MBA, Mouse bioassay; MHC II, Major histocompatibility complex class II; MPN, Mice phrenic nerve; MRM, Multiple reaction monitoring; MW, Molecular weight; NAP, Non-toxic neurotoxin-associated protein; OPCW, Organisation for the Prohibition of Chemical Weapons; pAb, Polyclonal antibody; PEG, Polyethylene glycol; PSP, Paralytic shellfish poisoning; PT, Proficiency test; PTC, Progenitor toxin complex; QC, Quality control; RM, Reference material; SE, Staphylococcal enterotoxin; SNARE, Soluble N-ethylmaleimide-sensitive factor attachment protein receptor; SRM, Selective reaction monitoring; STX, Saxitoxin; TCR, T-cell antigen receptor; TSST, Toxic shock syndrome toxin.

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1. Introduction

By definition, biological toxins are molecules produced by living organisms that induce harmful effects in other organisms when taken up e.g. by inhalation, ingestion, injection, or absorption. Toxins can be produced by many different microorganisms, plants, and animals, e.g. as high molecular weight protein toxins or as low molecular weight substances [1]. Based on their characteristics, biological toxins reside between the classic biological and chemical agents: they are produced by living organisms, but are unable to replicate, therefore inducing non-communicable diseases. In that respect, they share characteristics of classic chemical agents. Especially protein toxins often exert an enzymatic function within their endogenous target structure, amplifying their potency. This clearly differentiates them from low molecular weight agents and, additionally, is the reason for their extraordinarily high specific toxicity to mammalians [2].

Biological toxins are relevant in the health and food sector as well as in the security sector [1,2]. On the one hand, many biological toxins are linked with natural intoxications and are the etiologic agents of human diseases, e.g. food poisoning induced by staphylococcal enterotoxins (SE), the life-threatening neuromuscular disease botulism induced by botulinum neurotoxins (BoNT), and paralytic shellfish poisoning (PSP) with its prototypic toxin saxitoxin (STX) [1]. Additionally, plant toxins such as ricin or abrin have been known for centuries to induce accidental or, rarely, intended intoxications [3,4]. On the other hand, the relative ease in preparing some of the mentioned toxins, the world-wide availability of the source organisms as well as the threat potential for a major public health impact has led to their classification as potential agents of bioterrorism [5,6]. In this context, the ricin-containing threat letters sent to the U.S. President and other decision makers received high publicity and clearly demonstrated that there are criminal individuals interested in producing biological toxins and in intentionally releasing them to harm other people and to create fear by building a threat scenario. Based on open information, there is also evidence that criminal or militant groups are focusing their attention on other toxins, such as abrin or BoNT, since individuals have been arrested trying to acquire toxins illegally and to plot a crime [3,7]. Additionally, various nations have explored some of the mentioned toxins for military purposes, e.g. ricin as “Agent W”, BoNT as “Agent X”, and STX stockpiles and suicide pills [8,9]. Therefore, toxins are explicitly regulated under the Chemical Weapons Convention (CWC; ricin and STX are listed as Schedule 1 compounds of the CWC, Table 1) and/or the Biological Weapons Convention. Based on their relevance in different sectors, this review covers the current status of detection of biological toxins that carry a potential bioterrorism risk.

2. Challenges in detection of biological toxins

Since biological toxins are a source of natural intoxications as well as potential agents of bioterrorism, it is important to know how well prepared our open societies are with respect to their detection. In a potential bioterrorism or similar threat scenario, adequate management decisions rely on correct and reliable analytical results. A high level of analytical capabilities sets the basis for understanding the scale of the incident and for qualified decisions on countermeasures. However, in the absence of regular training opportunities it is rather difficult for laboratories to self-evaluate their analytical performance. In this context, the European Union funded the project EQuATox (“Establishment of Quality Assurances for the Detection of Biological Toxins of Potential Bioterrorism Risk”, [10]) from 2012 to 2014. The main task of EQuATox was to define the *status quo* of detection technology for biological toxins of potential bioterrorism risk by networking 35 expert laboratories from 20 countries [11]. Recent findings of EQuATox have shown that the equivalence of analytical results from different laboratories needs further improvement and that it is important to define basic quality requirements for biological toxin detection and identification [11].

Although biological toxins induce many different effects in the human body, they share some commonalities. From a diagnostic point of view, their detection is a challenging task for four basic reasons:

(i) Biological toxins are still poisonous in the absence of the producing organism and its genetic information. Therefore, the focus of detection cannot be – as in the case of pathogens – on nucleic acid-based methods such as polymerase chain reaction or sequencing technologies. Rather, the toxin itself has to be detected either by spectrometric, immunological, chromatographic, and functional assays or combinations thereof [2].
(ii) Due to the high specific toxicity, detection limits in the one to two digit pg/mL range have to be reached for certain protein toxins in order to detect poisonous doses. This is only achieved by selected technical approaches, especially when complex sample matrices are being analyzed.
(iii) Toxins are rapidly metabolized and degraded after incorporation, limiting the time window for successful identification and forensic analysis.
(iv) Most importantly, the precise detection and identification of biological toxins is hampered by the fact that they occur naturally in multiple isoforms or variants that may or may not vary in terms of toxicokinetics and toxicodynamics (see 2.1. to 2.4.).

Currently, expert laboratories use many different technical approaches for detection, identification and quantification of biological toxins. Valuable in-house validation studies have been published, presenting results obtained using individual technical approaches. However, hardly any universally agreed reference (“gold standard”) methods are available, nor are there certified reference materials (CRMs) for most of the toxins in focus (ricin, SE, BoNT). In the absence of CRMs expert laboratories use in-house purified materials or commercial toxin preparations of varying quality as quality control samples or for calibration, which makes objective comparison of accuracy and sensitivity of different methods questionable. In this situation, EQuATox started to work on basic quality requirements for toxin detection, focusing on the generation and characterization of reference materials (RMs) for spiking of samples and the organization of large international proficiency tests (PT), taking into account detection of different levels of toxin spiked into buffer and complex environmental, clinical, or food matrices [11]. By definition, PTs are performed to evaluate the participants’ performance against pre-established criteria by comparing their measurement results with assigned values and with results obtained in other laboratories. In this type of interlaboratory study, the participants are generally free in the choice of the analytical method that should be consistent with their normal routine practice [12,13]. This approach is usually performed prior to method-performance studies assessing the characteristics and performance of individual methods.

2.1. *Ricin from* Ricinus communis

Ricin is produced by the castor oil plant *Ricinus communis* and belongs to the larger family of type II ribosome-inactivating proteins. The toxin is an *N*-glycosylated protein consisting of a cell-binding B chain and an enzymatically active A chain, both linked by a disulfide bond forming a protein of about 63 kDa [4,14] (Table 1 and Fig. 1A). Upon uptake into the human body, ricin binds via its B chain to galactose and *N*-acetylgalactosamine moieties on

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