Yesterday, Today, and Tomorrow: A Selective View of Toxins in Weapons and Medicine

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Abstract

A look at the past and a look at the future. What is the history of toxins used as weapons and where is the danger in the future?

Before 2001, there was recognition that biological weapons, including toxins, were weapons that required state sponsorship. But that is no longer true. As the asymmetries of weapons and politics have changed, so has the probable use of toxins as weapons. Our understanding and expectation of likely use of toxins in weapons has changed. Even the word toxin is commonly used inappropriately to mean toxic chemical rather than a chemical produced by organisms. The possibility of toxin use in weapons was evaluated on whether the toxin could be manufactured or purified from natural resources. The more complicated the toxin's structure, the less likely it was to be purified or created using laboratory synthesis. Many infectious disease specialists regard toxins as the eventual cause of the destruction of cells in most bacterial infections.

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In the spectrum of chemical and biological weapons, from the simple chemical structures of mustard and sarin to the complicated toxins, such as palytoxin, where do toxin weapons fit? Does the use of toxins as a weapon imply the use of biological weapons or, if the toxin is laboratory synthesized and purified is it a chemical weapon? Do toxin weapons need to have purified chemicals? Are toxins the ultimate dual use agents? Do toxinologists need to examine their research and publications for the potential of possible illicit use? Do restrictions on research and publication of biologically based materials, including toxins, really impede the development of weapons? How can these restrictions be implemented to guarantee security concerns?

Introduction

Prior to 2001, most people believed the manufacture and use of toxins and biological weapons required state sponsorship because of the technology and equipment to prepare and purify the agents. That is no longer true. Now it is generally recognized that agents do not need to be pure and they certainly can be prepared from a variety of sources using many different methods, including those that have been developed from new and evolving technologies.

The use of asymmetric weapons has enabled small groups and even individuals to threaten large countries. Biological weapons and toxins can be used to make political statements as well as cause inordinate amounts of defensive or protective measures. The expectation of the use of toxins in weapons has changed; a little bit of agent can go along way and have a considerable influence on politics. The media and politicians have made the use of the term toxin so common as to even dilute its meaning; now the word toxin is commonly used to be a toxic chemical rather than a chemical produced by an organism.

A toxin is defined in the Merriam-Webster Dictionary as

> a poisonous substance that is a specific product of the metabolic activities of a living organism and is usually very unstable, notably toxic when introduced into the tissues, and typically capable of inducing antibody formation. (Merriam-Webster)

Prior to the new developments in genetic engineering and protein synthesis, the possibility of using a toxin in weapons was evaluated on whether the toxin could be manufactured or purified using natural materials. In today's world, the laboratory synthesis of toxins is commonplace and not necessarily more complicated by the structure of the toxin.

Concerns about the use of chemical and biologically-based weapons have taken on new urgency and significance as politics and dwindling resources have pushed more societies into the use of asymmetrical warfare. In many ways both chemical and biologically-based weapons are the perfect instrument for smaller entities (individuals or groups) to either defend or attack themselves. In efforts to defend and preserve life, many organisms have evolved to make toxic chemicals, toxins, and ways to disperse them for use against predators or to disable their prey to gain an advantage.

> In their struggle for life some animals grew fangs, others claws or tusks, while still others produced poisons. – Szent-Gyorgyi, Albert (M.D., Ph.D., Nobel Laureate for Medicine), The Crazy Ape, The Universal Library 1970

Humans have used these toxins developed by other organisms, e.g., plants, jellyfish, insects, or even mammals, for thousands of years, and humans have become quite adept in their use both for weapons and for medicines. At each new stage of technology, scientists have needed to evaluate the foreseeable uses of that technology, whether for good or bad. In many cases, the use of technology against society is an unintended or unforeseen consequence. The world is again at a stage for evaluations and this article encourages scientists and politicians to pause to think of the future and the role of counterbalances to regulate or curb further possible illicit and destructive use of toxins. The history of toxin studies and many of the facets of toxins are well presented in various chapters in this Handbook of Toxinology. Biological Toxins and Bioterrorism.

A Selective History

History abounds with examples of toxins used as weapons, and in most cases, the organism producing the toxin is included so that the history of toxins is also the history of biological warfare (see for example Globalsecurity.org and Christopher 1997). Humans have sought ways to gain advantages over their enemies for centuries, including using biological weapons as direct as hurling live poisonous snakes over walls and dumping dead and decaying cows and horses in wells and rivers. More sophisticated uses of biological weapons included dipping conventional weapons in material known to convey diseases (e.g., feces from populations infected with cholera). Some cultures used snake venom, venom from frogs, and even toxins from sea weed to make their swords, spears and arrows more effective. A selected list of toxins and venoms, which are believed to have either been used as weapons, including hunting, sabotage, assignations, etc., or could be used is presented in Table 1. These toxins include some of those naturally occurring naturally in plants (Magnuson (1997)).

Simplistically, toxins are proteins produced by animals, plants and bacteria and venoms are usually a mixture of toxins (Globalsecurity.org). Most readers already know that toxins are produced directly by organisms or indirectly, meaning they are metabolic side products, whose production is often increased in response to environmental conditions. The discussion of whether toxins are chemical or biological agents colors the history of toxins as weapons and the perception of their use.

In 1975, the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction (1972), otherwise known as the BTWC, entered into force (BTWC). It was an improvement on the 1925 Geneva Protocol, which prohibits the use of chemical and biological weapons in war. According to the US Code,

Table 1 ***Some possible bacterial, fungal, plant and animal toxins in weapons***. This table contains some toxins that have historically been used in weapons, including hunting. The list also contains some toxins considered to be possible to use in weapons. The list is not a complete list of all toxins. When possible the type of chemical, the active biochemical site and possible biological source, plant, fungi, bacteria, and animal are noted

Toxin	Type of chemical	Active site	Biological source
Ricin	Carbohydrate-binding protein	Inhibits ribosomal protein synthesis	Castor oil plant, *Ricinus communis*
Saxitoxin (paralytic shellfish toxin)	Nonprotein toxin	Neurotoxic alkaloids block Na and K channels in nerve cells	Marine dinoflagellates
Maitotoxin, palytoxin, ciguatoxin,	Nonprotein toxin	Disrupt ion channels	*Gambierdiscus toxicus*, dinoflagellate
Tetrodotoxin	Nonprotein toxin, aminoperhydroquanizole	Blocks Na channel	Bacteria, *Pseudoalteromonas tetraodonis*
Botulinum toxins	Single polypeptide chains	Blocks release of acetylcholine	*Clostridium botulinum*
Clostridium perfringens toxins	Proteins	Toxins specific to intestinal cells	*Clostridium perfringens*
Staphylococcal enterotoxin B	Protein	Toxin specific to intestinal cells	*Staphylococcus aureus*
Anthrax toxins	Protein	Disrupt cellular signalling	*Bacillus anthracis*
Plague toxins	Protein	Disrupt immune cells	*Yersinia pestis*
Aflatoxin	Difuranocoumarins	DNA damage, carcinogenic and protein inhibition	Fungi, e.g., *Aspergillus flavus*
Tricothecene	Fused ring compounds	RNA damage	Fungi, e.g., *Fusarium* and *Stachybotrys*
Snake venom	Proteins and polypeptides	Disrupt various biochemical processes; cytotoxins and neurotoxins	Many snake varieties, including cobras, rattle snakes, vipers, and sea snakes
Scorpion venom	Proteins, peptides,	Neurotoxins, channel blockers, enzyme inhibitors, etc.	Arthropods, including Brazilian scorpions, Emperor scorpions, Deathstalker scorpion
Spider venom	Proteins, peptides, polamines and other substances	Neurotoxic and cytotoxic (necrotic)	Arthropods, including Black widow, brown recluse and

> the term 'toxin' means the toxic material or product of plants, animals, microorganisms (including, but not limited to, bacteria, viruses, fungi, rickettsiae or protozoa), or infectious substances, or a recombinant or synthesized molecule, whatever their origin and method of production, and includes – (A) any poisonous substance or biological product that may be engineered as a result of biotechnology produced by a living organism; or (B) any poisonous isomer or biological product, homolog, or derivative of such a substance; (USC 2012)

The BTWC defined toxins as including "(both proteinaceous and non-proteinaceous) of a microbial, animal or vegetable nature and their synthetically produced analogues." In those intervening years between 1925 and 1972 and 1975, science had advanced so that toxins that were originally considered biological products could also be produced in the laboratory. In 1990, Dr. Graham Pearson described the CBW Spectrum, which illustrated the range of people potentially affected varied from chemical agents used in weapons through bioregulators and toxins to genetically modified biological warfare agents, and thus to the traditional biological warfare agents, such as anthrax and plague (Pearson 1990).

By the time the Chemical Weapons Convention, CWC, entered into force in 1997, discussions sometimes wandered over whether or not toxins purified from biological sources should be considered toxins or a chemicals, or were chemicals only those produced synthetically in the laboratory (CWC) With advances in genetic engineering and cell cultures, such discrimination is pointless from a technology viewpoint but still perhaps useful from a political perspective and as a negotiation tool.

Toxins in Nature

Types of Toxins

Most toxins are proteins and their toxicity depends on disrupting one or more biochemical pathways necessary to metabolism. Toxins are very selective about the biochemical pathways they disrupt. This selectivity also means only a very small amount of toxin needs to be present, but it must be delivered to the point where the disruption can occur. This usually means the toxins must be delivered safely to the bloodstream and preferably avoid the digestion systems unless they are stable.

Most proteins are not very stable; they react with water, oxygen, light, and heat. They also react to changes in pH and with other chemicals. The effectiveness of toxins in nature depends on their delivery. Organisms using toxins for defense may inject toxins under the skin or in the blood system with teeth or barbs. They may rely on spraying the toxin in a concentrated liquid into eyes or other mucosal tissues so that the material can be easily transferred to the bloodstream. Many toxins are destroyed during digestion or diluted or reacted in water.

Animal toxins include neurotoxins, toxins that interrupt the transmittal of nerve signals, cardiotoxins and cytotoxins that act on membrane lipids/proteins of blood cells or heart cells or produce severe arrhythmia of heartbeat, myotoxins that act on muscle cells, hemotoxins that affect the coagulation properties of blood, and vasoactive toxins that affect blood pressure.

The list of plant toxins is large and includes abrin, ricin, hemlock alkaloids, lectins, urushiols, cyanogenic glycosides, digitoxins, and fungal toxins (mycotoxins, tricothecenes, aflatoxin, etc.). Reviews in this Handbook include abrin (an toxic protein derived from the seeds of the plant Abrus precatorius), aflatoxin (mycotoxins that are produced by Aspergillus flavus), mycotoxins in general and ricin.

Bacterial toxins include exotoxins, secreted by the bacteria, and endotoxins, those parts of the cell structure included in the cell envelope. Most bacterial toxins are exotoxins, e.g., botulinum, shiga, tetanus and diphtheria toxins. Endotoxins are defined as the lipopolysaccharide (LPS) complex associated with the outer membrane of Gram-negative pathogens such as Escherichia coli. The LPS complex can interact with the immune system of monocytes and macrophages to induce releases of cytokines and eventually produce toxic shock.

Aptamers, single-stranded DNA and RNA proteins originally produced by both plants and animals, but subsequently engineered and grown in vitro, are used to target specific molecules and pathways. Although not necessarily fitting the classic definition of a toxin, these chemicals can be made to be much more stable than the original proteins and still offer specificity and discrimination as targeting drugs (Al-Shamy 2009; Toxin Targeting 2008). The specific targeting and interference with biological pathways conforms to more modern biotechnology definitions of toxins and may also be referred to as bioactive peptides.

Release or Use of Toxins

In nature, the organism generally releases a toxin when it is threatened, incapacitates its prey, or, as an aid in digestion. Scorpions, snakes, and many other animals release the toxins in a barb or sharp tooth that is hollow and delivers the toxin by breaking through the skin or shell of the attacker. Jellyfish use nematocysts, capsules containing toxin and a very small barb (Brinkman 2007; Brinkman and Burnell 2007). Some frogs and amphibians release toxins through their own skin into the skin or mucous membranes (mouth and gastrointestinal tract) of the attackers. As spiders bite into the attacker or prey they release venoms containing toxins as a digestion aid. Many animals use venoms containing a mixture of toxins and additives to help the toxins effectively reach their targets. Some snakes may spit into the eyes or mucous membranes of their attackers. All of these releases involve intimate contact between the organism and its attacker using a mode that breaks through natural defenses of skin and mucosal membranes. With the exception of ingestion, few natural methods of releasing toxins are passive; they require direct contact to be effective.

Bacterial toxins are released after infection by the bacteria. These toxins weaken the bacterial host so that the bacteria can more easily thrive and reproduce (Todar). Mycotoxins released from molds and fungi probably have similar roles to help breakdown the infected plants, but the releases of mycotoxins are perhaps more complicated and may be triggered by stresses in the environment. Plants and algae that produce toxins have likely evolved to use their toxins for protection. Ingestion of plants and other organisms containing toxins requires the attacker to eat the toxin containing material. Bacillus thuringiensis, Bt, a bacterium found in many soils, produces crystal proteins, Cry toxins, which interfere with the normal gastrointestinal activities of many invertebrates eventually killing them. The attackers learn avoidance; those that do not eat that organism do not get sick, or only those attackers immune to the toxin survive.

The effectiveness of toxins depends upon the biochemical disruption they cause, but a key part of their effectiveness is the delivery to specific target pathways. Without an effective delivery, most toxins will react and decay so that less material reaches the target. Extracting a toxin from an organism and then putting it in water more often than not destroys the toxin. In more military terms, organisms using toxins must be able to weaponize the toxin for effective delivery. Whether for an individual plant or animal, synthesizing the toxin is only the beginning of the creation of a weapon: the extraction of the toxin and the delivery system are crucial components.

The Dual Nature of Toxins in Medicine and as Weapons

As human understanding of biology has grown, the science of toxins has been used in medicine to treat diseases and conditions. Examples of medicinal use of toxins include bacterial, animal and plant toxins. Botulinum and other toxins are well known to cause paralysis, and their specialized use via injections can prevent muscle spasms and smooth wrinkles. Anti-coagulants components of snake venoms can be used to treat and prevent blood clots (Rojnuckarin 2013). In another type of usage, Bt is used as an insecticide to protect plant crops.

Using genetic modifications, scientists are learning to select and modify genes to enable organisms to be less susceptible to toxin disruption. The converse is that the knowledge also can work to identify organisms more susceptible to toxin disruption. Genetic selection can be used to select drugs and biochemical pathways unique to individuals and groups to improve drug delivery and effectiveness. But, the same techniques can be used to improve toxin delivery and effectiveness when toxins are used as weapons.

Advances in science can be used to develop new methods and refine existing methods that deliver toxins to the bloodstreams of both animals and humans. Weaponizing a toxin can use the same methods to develop mass inoculations or quick and easy delivery of medicine. The fastest and most effective route to deliver medicine is often by inhalation; the drug can quickly enter the bloodstream because very thin walls separate the blood capillaries and the alveoli of the lungs. This works well with vaporized and aerosolized materials. Proteins (or vaccines) attached to nanomaterials can be delivered quickly via inhalation and then once in the bloodstream, these same materials can be used to speed toxins to their targets.

Technologies using viruses to deliver chemicals directly into tumors may also be used to target specific organs and bypass protective barriers or components of immune systems (Al-Shamy 2009). What works well in drug delivery, works well in toxin delivery. The same science used for defense and protection can be used to improve weapons.

Historically, using toxins as weapons (or for defenses, depending on perspective) does not need pure chemicals. Using toxins as weapons can be elegant or crude. Putting corpses in wells or upstream of towns or throwing snakes over city walls are crude uses, but effective. The more elegant, purified toxin weapons are really most effective as sabotage or terror weapons because the delivery system can be stealthier than the crude uses.

Toxins are at the nexus of many new techniques in biology, medicine, genetics, immunology, and nanotechnology. Toxinolgy can be thought of as the Janus of biology, a point where the beginning and changes in technologies cause scientists and societies to look back on their histories and look forward to the future. The study of toxinology necessarily looks at both toxicology and weapons.

Ethics Education

What can scientists, technologists and societies use as an aid to planning advances currently and in the future? The study of ethics and its application is often taught as a general philosophy. The application of ethics to the intersection of biology, technology and medicine, bioethics, is required in most medical schools (NIH, Bioethics Resources on the Web). However, ethics and bioethics are fundamental to societies even if it is not a designated course or training. It is also not unique to scientists, technologists, medicine, chemistry, physics or any science (Mazor 2012). A consideration of ethics, the benign or aggressive use of toxins, is more obviously critical to toxinology than many other sciences or perhaps toxinologists are more aware of these issues because of the history of toxins. Dual-use is a part of nature and the evolution of toxins. Most organisms make and use toxin for defense and survival.

An important point in this discussion of history and future of toxins is not who or what makes a toxic chemical, or what can improve a weapon, it is “Are societies imposing limits on the development and research in technology in order to solve political problems?” How are decisions made on which weapons are banned? Who decides which characteristics of the weapons determine such bans? The processes used to discuss these issues are not easy ones and the discussions need to involve both scientists and policy makers. However many times the participants in these discussions do have not the training required to understand the implications of the policies or the science.

It is incumbent on science education programs to include ethics (Green 2013) As in medicine, the creed of “first do no harm” should be the pivotal question when applying technology or conducting research. It should also be the pivotal question in policy making. Ethics and biomedical ethics are often taught as part of medical training. Indeed most publications in ethics focus on the ethics in medical treatment and clinical practice. There are few programs in ethics for general science.

In the US, review boards evaluate studies proposed by scientists for dual-use potential in research. This is a time-consuming process that gives a false sense of security to those research projects successfully passing the review (US DURC 2012, 2013) In Europe, Dutch authorities recently ruled that publication of research by virologists in an international journal required an "export license" according to EU law and this now applies to 28 countries. Australia has suspended its Defense Department review of research on special pathogens while researchers and law-makers discuss how to modify their reviews so that science is not hindered but security is not deterred (MacKenzie 2013). A more general training in ethics in science, engineering and political studies may help to streamline this type of review. Indeed the need for communication between scientists, technologists, policy makers and lawyers has increased as progress and advances in science and technology have grown. At the same time the gap in understanding between science and non-science has widened.

Conclusion and Future Directions

In modern societies where success often includes rapid applications of new technologies, the recognition of the importance of reviewing the technologies against a set of ethics can be a new concept. Who determines the set of ethics, the guidelines for the reviews, and the process? How can this process be implemented? Is the process really needed? How can the processes be structured so that they do not impede progress?

There are few advances in science or technology that cannot be turned for malevolent use. Szent-Gyorgyi, a Nobel Prize winning anti-war scientist, isolated vitamin C (NLM). Certainly this was peaceful tool used to alleviate malnutrition? Yet, the Nazis used vitamin C to enable their ships to stay at sea longer. This is one example of how scientists cannot control or anticipate illicit or compromising uses of technology and science, even peaceful uses. Would a review board of scientists have considered the isolation and synthesis of vitamin C a potential dual-use area of research?

A review of the history of toxinology, pharmacology and even defense science emphasizes that training in ethics needs to begin early, at least in undergraduate science programs. The importance of ethics is not reserved for medical doctors or graduate programs or even scientists. Engineers and technicians with bachelor degrees are often very capable of applying and changing technology. And everyone in society participates in the use of technology, whether they study science or use results of technology. Ethics training should be for every individual.

Cross-References

▶ Botulinum Toxin: Present Knowledge and Threats
▶ Evolutionary Traits of Toxins
▶ Marine Biotoxins in History: Misuse and Mayhem

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