Original article

Threat of terrorism by using chemical and biological warfare agents: Bioluminescence assay as a military biodefense

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Abstract

Background : There is increasing risk of the proliferation of biological and chemical warfare agents with recent advances in science and technology, especially involving the integration of chemistry and biology. Exacerbating this condition is the fact that the high tech development of more advanced non-lethal chemical/biological weapons appears to be generally tolerated.

Method : Literature review on chemical and biological warfare agents, biochemical assay, and the British Broadcasting Corporation (BBC) news about the anthrax-related deaths of hippos.

Results : (1) Many commercial automated biochemical test platforms are today available for performing the tests after growth in bacteriological media.

(2) The polymerase chain reaction (PCR) based assays can detect >10 microorganisms per sample but limitations are with the detection of toxins and other non nucleic acid containing prions, etc.

(3) The bioluminescence has been widely used mainly in the quality control testing of bacterial contamination in food industry.

(4) The antigen and antibody based immunoassays are being developed for the detection of bacterial and viral biowarfare agents.

(5) BBC news announced that More than 100 hippos have been found dead in a Namibian national park, with authorities suspecting outbreak of anthrax could be to blame on 9 October 2017, denying possibility of terrorist's attack.

Conclusion : In conclusion, it is very much important for us to develop specific diagnostic, therapeutic capabilities and capacities alongside training and education and also, we will have investigate process for specific diseases outbreaks, as well as mitigating the effects of terrorist attacks by using chemical warfare (CW) agents and biological warfare (BW) agents.

Key words

terrorism, anthrax, bioluminescence based detection

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1. Introduction

There is increasing risk of the proliferation of biological and chemical warfare agents with recent advances in science and technology, especially involving the integration of chemistry and biology [1~3]. Exacerbating this condition is the fact that the high-tech development of more advanced non-lethal chemical/biological weapons appears to be generally tolerated [2~5]. Although the non-lethal weapons are defined as the ones which 'have a reversible effect on their human targets', the term non-lethal weapons is a misnomer as there will always be an element of risk associated with the use of any weapon system. Historically, these weapons have been classified by their effects, principally whether they disable, disorient, discourage, demobilise or deceive victims. The medical effects of non-lethal weapons may be broadly categorised into:

- blunt trauma effects
- eye effects
- auditory effects
- electrophysiological effects
- toxicological/pharmaceutical effects
- psychological effects

Therefore, we describe significant demand for devices that can rapidly detect chemical–biological–explosive threats on-site and allow for immediate responders to mitigate spread, risk, and loss. And also, the Centers for Disease Control and Prevention and United States Army Medical Research Institute of Infectious Diseases have developed real-time polymerase chain reaction (PCR) assays for the detection of bioterrorism threat agents. The key to an effective reconnaissance mission is a unified detection technology that analyzes potential threats in real time. We also describe an outlook toward future technologies, and describes how they could possibly be used in areas such as war zones to detect and identify hazardous substances.

2. Detection for chemical warfare (CW) agents

Toxic chemicals are defined as any chemical which can cause death, temporary incapacitation, or permanent harm to humans or animals, through its chemical action on life processes, whilst precursors are the chemicals involved in the production of toxic chemicals [6]. Munitions or devices include such things as mortars, artillery shells, missiles, bombs, mines or spray tanks. Hence they are specifically designed to inflict harm or cause death through the release of toxic chemicals [6].

It is well known that chemical agents (CAs) have been used against military personnel during conventional warfare, however, due to the increasing threat of terrorist activities, the focus has now broadened to encompass the threat posed to civilians [6]. As a result, the perceived threat of a CA attack has the potential to create great panic in parties that are unprepared because when released, these agents are amorphous and not able to be evaded [6]. Hence, if there is no capability available to detect and monitor these agents it is safe to assume that there is no preparation for a potential attack, and therefore the first signs of exposure to an agent will be when symptoms begin to appear, which may be too late [6].

And also, the effectiveness of a particular detection technology can be a function of the chemical's physical properties and although the technologies have progressed significantly, there is still room for improvement. The major challenge is the need to increase detection reliability and reduce the frequency of false alarms. The future direction for detectors is to develop a capability for the detection of not only chemical warfare (CW) agents but

also for a wide range of toxic industrial chemicals.

3. Detection for biological warfare (BW) agents

Among all the detection systems available for use, none of them satisfy all these criteria and the selection of methodology should be situation specific [7]. Development of detection systems that can detect the biological agents in concentration at which they can cause disease in humans is a challenge, and due to lack of sensitivity of many of the available antigen and antibody based systems, research is focused on development of nucleic acid based sensors that are much more sensitive but need complex sample preparation [7, 8]. The polymerase chain reaction (PCR) based assays can detect >10 microorganisms per sample but limitations are with the detection of toxins and other non nucleic acid containing prions, etc [7, 8]. For example, Prion diseases are usually diagnosed clinically and confirmed by post-mortem histopathological examination of brain tissue. The only reliable molecular marker for prion diseases is PrP(Sc), the pathological conformer of the prion protein that accumulates in the central nervous system and, to a lesser extent, in lymphoreticular tissues. For BSE, several commercial diagnostic kits based on the post-mortem immunochemical detection of PrP(Sc) in brain tissue are now available. Therefore, it is impossible for us to have PCR for diagnosis to PrP(Sc). Further, the nucleic acid based assays are to be performed in much cleaner environment as there is a possibility of DNA from laboratory and instrument contamination getting amplified and thus producing a false positive result [8].

3.1 Biochemical test based assays

The culture of bacteria in a routine microbiological laboratory and further identification based on biochemical tests is the conventional identification method mostly followed in most of the hospital setups even today [8, 9]. As this method is followed for various diseases, the biological warfare (BW) agents, mainly the bacterial agents, need to be identified through this platform on a routine basis, if not during an intentional outbreak, but during natural prevalence of the disease [8, 9]. Many commercial automated biochemical test platforms are today available for performing the tests after growth in bacteriological media, but these systems are mostly developed for bacterial diseases that are clinically important [9, 10]. Therefore, we have proposed that from now on, Biochemical test based assay will be developed for terrorism.

3.2 Bioluminescence based detection

Bioluminescence is the monitoring of luciferin and luciferase, the enzyme, interaction in the presence of adenosine triphosphate (ATP). The basic principle is ATP is found in all the living cells like virus and bacteria and its amount corresponds to the microbial load in air, water and in other environmental samples. The bioluminescence has been widely used mainly in the quality control testing of bacterial contamination in food industry. The continous air quality monitoring will trigger alarm for any unusual raise in the microbial load in the environment. For example, Imamura et al. developed specific assay of bioluminescence based detection [10]. Allele-specific PCR for E. coli O157 was conducted with primers specific to verotoxin genes, verotoxin 1 (VT1) and verotoxin 2 (VT2). VT is an important cause of haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) worldwide [10]. They developed a simple, rapid bioluminescent detection method for E. coli O157 [10]. The method is based on the determination of pyrophosphoric acid (PPi) released during allele-specific PCR.

Thus, released PPi is converted to ATP by ATP sulfurylase and the concentration of ATP is determined using the firefly luciferase reaction [10]. As a result, VT1, VT2 and DNA with VT1/VT2 were clearly identified by this method [10]. This protocol, which does not require expensive equipment, can be utilized to monitor the PCR product rapidly [10]. Additionally, this methodology can be used as a high-throughput approach for measuring PCR products [10].

3.3 Antigen and antibody based detection systems

The antigen and antibody based immunoassays are being developed for the detection of bacterial and viral biowarfare agents. Arakawa et al. developed a novel bioluminescent assay of alkaline phosphatase (ALP) utilizing ATP sulfurylase and the luciferin–luciferase reaction [11]. The principle governing the assay is as follows. Adenosine-3' phosphate-5' -phosphosulfate, which serves as the substrate for ALP, is hydrolyzed enzymatically to produce adenosine-50-phosphosulfate (APS) [11]. APS is converted into ATP by ATP sulfurylase in the presence of pyrophosphate [11]. The ATP produced is detected by the luciferin–luciferase reaction [11]. The measurable range was 1 zmol to 100 fmol/assay and the detection limit at blank+ 3 SD was 10 zmol/assay [11]. The coefficient of variation (CV, n=5) was examined at each point of the standard curve; the mean CV percentage was 4.47% (n=6). This assay system was applied to enzyme immunoassay of human chorionic gonadotropin and allele-specific PCR enzyme-linked immunosorbent assay of verotoxin gene using ALP as the label enzyme; 10^{-2} mIU/mL hCG in urine and 5 pg of Escherichia coli O157 DNA could be assayed directly and with high sensitivity by the proposed method [11].

4. Discussion

Terrorists want to have an approach likely to be the most cost-effective and also, the likelihood of a successful terrorist attack is not very large, given the technical difficulties and constraints resulting from the need to work in secret, and more probably at the low-technology end of the spectrum than the high-technology end that is shown in table 1 [5, 12, 13].

Recently, the British Broadcasting Corporation (BBC) news announced that more than 100 hippos have been found dead in a Namibian national park, with authorities suspecting anthrax could be to blame on 9 October 2017 and also, the first hippo was spotted on October 1 according to acting director of Namibia's Ministry of Environment and Tourism, Johnson Ndokosho. Since then, at least 100 have turned up dead in the western region of Bwabwata National Park, which sits in a northeastern Namibian strip, sandwiched between Angola and Botswana [14]. Namibia's Ministry of Environment and Tourism suspects that there was an anthrax outbreak because the river is very low under light rain due to weather condition of this year so that we can guess that the outbreak of anthrax has no relation with terrorism. However, it shows us the possibility that we may encounter a very serious issue with domestic animals being attacked by CW agents or BW agents by terrorists.

Therefore, it is very much important for us to develop an assay system for diagnosis to CW agents or BW agents by using bioluminescence based detection with high sensitivity and high-throughput.

As for developing a therapy for anthrax, through our experience, lions that are flesh-eating animals can survive after eating other animals with anthrax. However, nobody knows the reason why the flesh-eating animals can survive so this gives us the motivation to investigate the disease's clinical

Table 1 Main agents potentially involved in specific issues by terrorists

Disease	Agent	Organisim Persitence	Human to Hunam transmisson	infection dose	treatment	Incubation period	Mortality
Staphylococcal enterotoxin B	Produced by Staphylococcus aureus	Resistant to Freezing, inactivated at 100℃	No	0.03 μg/peron	supportive treatment	Inhalation 3-12 h ingestion 4-10 h	< 1%
Ricin	Derived from beans of caster plant Ricinus Communis	Stable until heated above 80℃	No	LD 50 1 mg	supportive treatment	Inhalation 4-8 h (mild symptoms) 18-24 h (severe symptoms)	High
Botulism	Botulinum toxin produced by Clastridum botulinum	Weeks in non-moving food or water	No	LD 50 is 0.001 μ/kg for type A (parenteral), 0.003 μg/kg (aerosol)	supportive treatment trivalent or heptavalent antitoxins	2 h to 10 days (mean 12-72 h)	Without supportive treatment; high mortality resulting from to respiratory failure
Anthrax	Spores of Bacillus anthracis	Very stable, Spores my be viable for 40 > years in soil	No	8,000-50,000 spores	Ciprofloxacin or doxicycline	1-6 days	High
Brucellosis	Genus Brucella	6 weeks, In dust to 10 weeks In soil or water	No	10-100 organisms	Doxicycline + refampicin	5-60 dyas	5% if untreated
Glanders	Burkoholderia mallei	Very stable	Rare but possible	Unknown	Ceftazidime, imipenem or meropenem	10-14 days	Very high if untreated
Melioidosis	Burkoholderia pesudomallei	Very stable	Rare but possible	Unknown	Post- exposure prophylaxis with co-trimoxazole	10-14 days	Very high if untreated
Plague	Yersinia	Up tp 1 year in soil, but viable only for 1 h after aerosol release	High	100-20,000 organisms	Streptnycin or gentamycin with ciprofloxation or doxycyvline	1-6 days	Very high if untreated, < 10% antibiotics
Q fever	Coxiella burnetii	Resistant to heat and drying, pressists for weeks to months	Rare but possible	1-10 organisms	Teracycline or doxycyline	7-41 days	1% untreayed, chronic form 30-60%
Salmonellosis	Genus salmonella	Resistant to heat up to 57-60℃	Faecal - oral transmission	Unknown	Supportive care to prevent dehydration. In severe infections fluoroquinolomes or third-generation cepharosporins	6-48 h	< 1%
Tularaemia (rabit fever)	Francisella tularensis ssp tularensis	Weeks in water, soil or caracasess and years in frozen meat	No	10-50 organisms	Streptnycin or gentamycin	1-25 days (mean 3-5 days)	4-50% withouut treatment
Small pox	Variola virus; Variola major	Higly stable for up to 1 year in dust and cloth	Yes, transmission requires close contcat	10-100 organisms	No antiviral treatment, vaccination immideately or up tp 4 days after exposure cab reduce mortality	4-19 days (mean 10-12 days)	Ordinary type Small pox 30% if unvaccinated; 3% if vaccinated
Shigellosis	Genous Shigella	Mean survivall of 2-3 days, up to 17 days in favoulable circumstances, sveral hours on infected hands	Fecal-oral transmission	10-100 organisms	Usuallly self-limiting. In severe infections, trimethoprim-sulphamethoxazole and ciprofloxacin shortain duration of symptoms and excretion in faces.	1-7 days	< 1%

pathology.

Anthrax is found all over the world. It contaminates the ground when an affected animal dies. It spreads when grazing animals pick it up from contaminated dirt or through contaminated food sources such as bone meal that may have been made from contaminated carcasses. There appears to be an increase in the cases of anthrax among grazing animals during droughts, when they tend to graze closer to the ground and consume more dirt with the grass.

Anthrax may also spread due to the bacteria which are then transferred to other areas by the host and contaminate the ground when that animal dies. As the animal decays, the bacteria are exposed to oxygen and turn back into the spores that contaminate the soil. The anthrax spores have a very tough outer casing and can remain viable in the ground for decades. Many diagnostic laboratories around the world have anthrax samples for use in research and for the identification of anthrax. Anthrax can be grown in laboratories from these existing spores. In the wrong hands, these spores can be grown, dried and milled for use in biological weapons.

Therefore, terrorist will have agents selected which are considered to be suited for causing mass casualties because they were found to share a number of characteristics.

And also, we can identify several dangerous chemical and biological weapons proliferation trends;

- Developments in biotechnology, including genetic engineering, may produce a wide variety of live agents and toxins that are difficult to detect and counter; and new CW agents and mixtures of CW agents and BW agents are being developed.
- Countries are using the natural overlap between weapons and civilian applications of chemical and biological materials to conceal CW agents and BW agents production; controlling exports of dual-use technology is ever more difficult.
- Countries with CW and BW capabilities are acquiring sophisticated delivery systems including cruise and ballistic missiles.

5. Conclusion

In conclusion, it is very much important for us to develop specific diagnostic, therapeutic capabilities and capacities alongside training and education and also, we will have investigate backbone for specific diseases outbreaks, as well as mitigating the effects of terrorist attacks by using CW agents and BW agents. Further investigation with respect to improvement of diagnosis and therapy of CW agents and BW agents is currently in progress. The Department of Military Science, University of Namibia was established in January 2014, but there are few Institutes of CW agents and BW agents in Africa. Therefore, further investigation with respect to the role of CW agents and BW agents in new diagnosis and therapy is currently in progress in our department.

Conflicts of interest

There is no identified association between the study conclusion and University of Namibia under conflicts of interest (COI). Therefore, the authors declare no COI.

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