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Of Beans and Beads: Ricin and Abrin in Bioterrorism and Biocrime

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Abstract

Ricin and abrin are among the most lethal plant toxins known to humans. Even minute amounts, if effectively used, could cause considerable injury and mortality. Because of potency, stability, relative ease of production, and worldwide availability of their source plants, ricin and abrin are potential biological weapons. Ricin was also developed as an aerosol biological weapon by the U.S. and its allies during WWII, but was never used in battle. Ricin is not considered an effective weapon of mass destruction, but it has been the agent of choice in numerous biocrimes. Despite being associated with death and biological warfare, both ricin and abrin are also well known for their use in therapeutic applications and biomedical research. This article provides an overview of ricin and abrin, and their perspectives in bioterrorism and biocrime.

Keywords: Ricin; Abrin; Ricinus communis; *Abrus precatorius*; Ribosome-inactivating proteins; Biological weapon; Bioterrorism; Biocrime

Abbreviations: RIPs: Ribosome-inactivating proteins; CDC: Centers for Disease Control and Prevention; BTWC: Biological and Toxin Weapons Convention; CWC: Chemical Weapons Convention; BWATA: Biological Weapons Anti Terrorism Act; VLS: Vascular leak syndrome; TRFIA: Time-resolved fluorescence immunoassay, PCR: Polymerase chain reaction; HPLC-ESI-MS: High performance liquid chromatography electrospray ionization mass spectrometry

Introduction

Abrin and ricin are potent phytotoxins isolated from the seeds of *Abrus precatorius* L. and *Ricinus communis* L., respectively. They belong to a family of plant ribosome-inactivating proteins (RIPs) which inhibit protein synthesis by inactivating ribosomes. Ricin and abrin are classified as Type 2 RIPs which include other phytotoxins such as modeccin (from the fruits and roots of *Adenia digitata*), volkensin (from the roots of *A. volkensii*), and viscumin (from mistletoe, *Viscum album*) [1]. Ricin and abrin share extensive identity and structural similarities. Both proteins have a molecular mass of approximately 60-65 kDa, consisting of two polypeptide chains: A chain and a larger B chain linked by a disulfide bond [1,2]. Their mechanism of action is similar: the B chain (galactose-binding lectin) binds to cell surface receptors to facilitate the transport of the toxin across the cell membrane, whereas the A chain, once internalized by the cell, acts on the 60s ribosome to inhibit elongation factor (EF)-1 and EF-2, preventing protein synthesis and leading to cell death [3-5]. Both toxins have been extensively characterized and their crystallographic structures were determined [6-11]. Although ricin is homologous to abrin, antibodies raised against ricin do not cross-react with those against abrin, and vice-versa [12].

The toxicity of ricin and abrin and their relative ease of production make them potential biological warfare agents or terrorists' weapons. Abrin and ricin have been classified by the Centers for Disease Control and Prevention (CDC) as Category B agents. Agents in this category are moderately easy to disseminate, and can cause morbidity and low mortality [13]. Ricin is currently monitored as a Schedule 1 toxic chemical under the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction (CWC). Both ricin and abrin are included in the latest version of the Biological and Toxin Weapons Convention Procedural Report and Rolling Text [14]. Additionally, the possession or transfer of ricin, abrin, or genes encoding functional forms of these toxins is also regulated in the U.S. by the CDC Select Agents and Toxins Program.

Origin, Distribution and Uses

Ricin toxin was discovered in 1888 as the first plant lectin from the seeds of the castor plant, *Ricinus communis* L. (Stillmark, 1888, as cited in [15]). *R. communis* (Euphorbiaceae) is indigenous to the southeastern Mediterranean region, eastern Africa and India, but is now widespread throughout temperate and subtropical regions [16]. It has been cultivated primarily for castor oil [17]. In Ancient Egypt, Europe, India and China, castor oil has been used for lighting, body ointments, purgative, cathartic and other ethnomedical systems. In Italy, castor oil was used as an instrument of coercion by the Squadristi, the Fascists armed squads of dictator Benito Mussolini [18]. Political dissidents and regime opponents were forced to ingest the oil in large amounts, triggering severe diarrhea and dehydration that frequently led to death [19]. During World War I (WWI), natural castor oil was first identified as a laxative and as a lubricant. Today, castor oil has a wide variety of commercial applications including medicinal and industrial purposes [20-24]. Because of its economic benefits and myriads of uses, castor seeds are currently being produced in more than 30 countries in the world with an annual production of more than 1.5 million metric tons; the leading producers include India, China, and Brazil [25].

Ricin can be made from the waste material left over from the processing of castor oil. It is stable under normal conditions, but can be inactivated by heat above 80°C. After oil extraction and inactivation of ricin, the defatted mash and seed husks are used as animal feed and

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fertilizer, respectively [26]. In the last decade, ricin has contributed to early immunology, the treatment of cancer, AIDS and other illnesses, and the understanding of cell biology. A common problem encountered in patients treated with ricin-based immunotoxins is the vascular leak syndrome (VLS), in which fluids leak from blood vessels leading to hypoalbuminemia, weight gain, and pulmonary edema [27].

Abrin is a toxin that is found in the seeds of *Abrus precatorius* L. (Fabaceae or Leguminosae). *A. precatorius* has aquired >30 common names, such as jequirity, rosary pea, rati or ratti, crab's eye, John crow bead, precatory bean, love bean, Indian licorice, akar saga, giddee giddee, or jumbie bead, and numerous other locally used common names, from its many uses. "Abrus" means beautiful or graceful and is used to describe the appearance of the seed; "precare" from which the species name is derived, means to pray, references its common name in rosaries. *A. precatorius* is native to Southeast Asia, possibly India [28] or of Guinea in Africa [29], but grows in tropical and subtropical areas of the world where it has been introduced. In the U.S., it is found in Alabama, Arkansas, Florida, Georgia, Hawaii, Puerto Rico and the Virgin Islands. *A. precatorius* is a perennial climber and has a tendency to become weedy and invasive where it has been introduced. The Florida Exotic Pest Plant Council's 2011 Record of Invasive Plant Species listed *A. precatorius* under Category I [30].

Though lacking the oil of castor seeds, the brightly colored *A. precatorius* seeds are popularly used as beads in rosaries and other jewelry and in toys or percussion instruments. In Trinidad in the West Indies, the seeds are strung into bracelets and worn around the wrist or ankle to ward off jumbies or evil spirits and "mal-yeux" (evil eye). The Tamils use *Abrus* seeds of different colors (red, black, white, and green varieties). Also, because of their uniform weight, *Abrus* seeds were formerly used in Southeast Asia for weighing gemstones; the Koh-i-Noor or mountain of light diamond belonging to the British crown, was reportedly ascertained by this means [31]. The Ganda system of weight was based on multiples of four *Abrus* seeds [17].

A. precatorius seeds, roots and leaves have a long history in ethnobotany and various preparations were used for a panoply of purposes such as an analgesic, aphrodisiac, abortifacient, anticonvulsant, laxative, sedative, insecticide, herbal remedy to treat chronic eye diseases, fevers, coughs and colds, worms, venereal diseases and other conditions [32]. All parts of *A. precatorius* plant are toxic, but the seeds contain the highest concentration of toxins. Poison absorbed through a prick on the finger when stringing for beads can also be a killer [33]. In India and Sri Lanka, seed extracts have been used to poison animals and humans [32-35]. Additionally, some African and Madagascar tribes used abrin as an ordeal poison [32].

Abrin can be made in several forms, i.e., powder, mist, pellet, or it can also be dissolved in water. Pure abrin is a yellowish-white amorphous powder, and like ricin, is very stable (Budavari, 1989). The medical potential of abrin for the treatment of cancers has been investigated. It was used with some clinical success as an analgesic in terminally-ill patients [36]. Ethanolic extracts of *Abrus* leaves were also demonstrated to possess d-tubocurarine-like neuromuscular blocking activity [37]. Further, abrin's use as a tool for research, e.g., cellular function, has been described [38].

History in Biowarfare

During World War I (WWI), ricin was investigated as a potential offensive biological weapon [39]. However, the thermal instability of ricin constrained its initial use in exploding shells and ethical and treaty issues limited its use as a poison or blinding agents (Hunt et al., 1918, as cited in [40]). The war ended before the toxin could be weaponized and tested. During WWII, ricin was produced, armed into W bombs (ricin-containing bombs), and tested, but apparently was never used in battle [15]. Interest in ricin continued for a short period after WWII, but soon subsided when the U.S. Army Chemical Corps began a program to weaponize sarin. During the Cold War, the Soviet Union also studied ricin as a possible biological weapons agent. Ken Alibek, a former top official involved in Russia's biological weapons program who defected to the U.S. in 1991, claimed that Russia developed ricin toxin as a weapon and that the ricin toxin used against the Bulgarian exiles Georgi Markov and Vladimir Kostov was concocted in Russian laboratories [41]. During 1989, ~10 L of concentrated ricin solution was manufactured in Iraq, some of which were used in animal testing and as payload in artillery shells [42]. A news item documented evidence of the manufacture of ricin and botulinum neurotoxin (BoNT) in Iraq [43]. Syria was believed to have produced unknown quantities of ricin. In 1992, Iran allegedly procured 120 tons of castor beans, presumably for ricin production [44]. In 2001, ricin was found in Afghanistan after the collapse of the Taliban government [45-46].

Even though ricin's potential use as a military weapon was investigated, its utility over conventional weaponry remains disputed. Despite its toxicity, ricin is less potent than other agents such as BoNT or anthrax. Kortepeter & Parker [47] estimated that eight metric tons of ricin would have to be aerosolized over a 100 km^2 area to achieve about 50% casualty, whereas only kilogram quantities of anthrax spores would cause the same effect. Furthermore, dispersal of ricin on a wide scale is logistically impractical. Thus, while ricin is easy to produce, it is not as likely to cause as many casualties as other agents [48].

Abrin is not known (to date) to have been used in any wars or terrorist attacks [49]. A number of abrin poisoning have been documented (see below).

Ricin Use in Terrorism and Biocrime

Though ricin is not considered an effective weapon of mass destruction, its potential as a weapon of terror cannot be discounted. The well-publicized "Umbrella Murder" of the Bulgarian dissident Georgi Markov on September 7, 1978 [50] represents the first case in recent history of state-supported terrorism with a biological agent [51], however, this remains unproven. Despite the KGB's denial, high-profile defectors Oleg Kalugin and Oleg Gordievsky have since confirmed the KGB's involvement [52]. Ten days before the assassination, an attempt was made to kill another Bulgarian defector, Vladimir Kostov, in the same manner as Markov, in a Paris metro station. Examination showed an identical pellet removed from his back [50]. Since then, numerous ricin incidents have been reported worldwide. The incidents below involved ricin and its use as a murder weapon, or as an espionage tool of assassination during the period 1981 to 2011.

In 1981, exposed CIA double agent Boris Korczak was reportedly shot with a ricin-laced pellet [53]. He survived this assassination attempt which was thought to be the work of the KGB.

In 1982, William A. Chanslor, a Texas attorney was fined and sentenced to jail for plotting to kill his wife with ricin [54].

In 1985, Montgomery Todd Meeks, a high school senior, was convicted of attempted murder and solicitation to murder in connection with a plot to kill his father using ricin (Trager, 1985, as cited in [53]).

In 1991, members of the Minnesota Patriots Council, a radical tax-

protesting militia organization, acquired castor beans and planned to use ricin to assassinate local deputy sheriffs, U.S. Marshals, and IRS agents. They were convicted in 1994 and 1995 under the Biological Weapons Anti-Terrorism Act (BWATA) law [55].

On April 21, 1992, the Washington Post published an article about the unsuccessful attempt to poison Soviet dissident Alexander Solzhenitsyn with ricin [56].

In 1995, Deborah Green, a non-practicing oncologist from Prairie Village, Kansas, attempted to murder her husband, Michael Farrar, a cardiologist, with ricin [53,57].

In November 1999, FBI agents apprehended James Kenneth Gluck in Tampa, Florida, for threatening to kill court officials in Jefferson County, Colorado with ricin [58].

In 2007, traces of ricin had been found at Limerick Prison (Lally, 2007). The ricin was smuggled into Ireland from the U.S. in a contact lens case, to be used in an assassination plot [55].

In June 2011, Michael Crooker, a former Agawam man, was sentenced to 15 years in prison for illegally possessing ricin and threatening a prosecutor [59].

A number of incidents involving ricin, its manufacture, and preparing acts of terrorism by extremist groups and individuals have also been reported. After the 9/11 attacks, incidents include the following:

In August 2002, the Sunni militant group Ansar-al-Islam was reported to have been testing biological weapons, including ricin, at a small facility in northern Iraq, experimenting on barnyard animals, and on at least one human [60].

In December 2002, six terrorist suspects were arrested in Manchester, England. Their apartment was serving as a "ricin laboratory." Among them was a 27-year-old chemist who was producing the toxin [61].

In January 2003, authorities arrested six Algerians in Wood Green, northern London whom they claimed were manufacturing ricin as part of a plot for a poison attack on the London Underground [62].

In October 2003, a package and letter sealed in a "ricin-contaminated" envelope was intercepted at a mail processing and distribution facility in Greenville, South Carolina. The letter was signed "Fallen Angel" and threatened to poison water supplies if demands were not met [63].

In November 2003, a letter postmarked Chattanooga, Tennessee, and addressed to the White House was intercepted [64]. The letter contained a fine powdery substance that later tested positive for ricin, which, investigators said was of low potency and was not considered a health risk [65]. Similar to the letter intercepted in October 2003, it was also signed "Fallen Angel," and complained about new federal trucking regulations requiring more rest for drivers.

In February 2004, traces of ricin were discovered on an automatic mail sorter in the mailroom of the Dirksen Senate Office building in Washington, D.C which handled mail addressed to Senate Majority Leader Bill Frist [66].

In January 2005, the FBI arrested an Ocala, Florida man after agents found ricin and other products in the home he lives in with his mother [67].

On October 3, 2006, a survivalist from Phoenix, Arizona was sentenced to 7 years in prison for attempting to manufacture ricin [68].

In November 2008, Roger Von Bergendorff was fined and sentenced to prison for possessing ricin and unregistered firearm silencers. In February 2008, authorities recovered castor beans, a weapons cache, a copy of "The Anarchist Cookbook" with a page about ricin marked, and 4 crude grams of ricin in Bergendorff's motel room in Las Vegas, Nevada [69].

In June 2009, a father and son, Ian and Nicky Davison were arrested after the discovery of ricin at a house in County Durham [70]. Ian Davidson, a British white supremacist and neo-Nazi, was sentenced to 10 years in May 2010 for preparing acts of terrorism, possessing material useful to commit acts of terrorism and possessing a prohibited weapon; his son was given 2 years youth detention for possessing material useful to commit acts of terrorism [71].

In January 2011, The FBI arrested the owner of a Coventry Township, Ohio home for unlawful possession of ricin [72].

In June 2011, a British citizen, Asim Kauser, was brought to court on charges including possessing instructions for producing ricin [73].

In August 2011, the U.S. government discovered information that terrorist groups were attempting to obtain large amounts of castor beans for weaponizing ricin [74].

On November 1, 2011, four elderly Georgia men (Frederick Thomas, 73; Dan Roberts, 67; Ray H. Adams, 65; and Samuel J. Crump, 68), were arrested relating to plans to obtain an unregistered explosive device and silencer, and to manufacture ricin for use in attacks against other U.S. citizens and government personnel and officials [75].

The above events clearly demonstrate that ricin is readily available or accessible, relatively easy to produce, and seemingly, a biological weapon of choice by extremist groups and individuals. Hence, it should be seriously considered as a potential bioterrorism threat agent.

Toxicity, Clinical Signs and Symptoms

Ricin's toxicity is dependent on a number of factors such as route of exposure (inhalation, parenteral (injection), ingestion, dermal contact, or ocular contact), amount of toxin administered, and animal species. The clinical signs, symptoms, and pathological manifestations vary with the dose and route of exposure. Most symptoms develop less than 6 hours after ingestion. Progression to death occurs within 36 to 72 hours of exposure, depending on the route of exposure and the dose received [76].

Ricin is extremely toxic by inhalation, and least potent by the oral route. In mice, the approximate dose that is lethal to 50% of the exposed population (LD_{50}) and time to death are, respectively, 3-5 μg/kg [15] and 60 hours by inhalation [77], 20 mg/kg and 85 hours by ingestion [15], 5 μg/kg and 90 hours by intravenous injection [15], and 24 μg/kg and 100 hours by subcutaneous injection [15]. Limited information is available regarding human toxicity. Based on animal experiments and accidental human exposures, the approximate LD_{50} and time to death for humans exposed to ricin from either inhalation, ingestion, intravenous, or subcutaneous administration of toxin have been reported [15,41,78]. Low oral toxicity is possibly due to poor toxin absorption and partial degradation in the gut.

In humans, subcutaneous or intramuscular injection of high doses of ricin results in severe local lymphoid necrosis, gastrointestinal hemorrhage, liver necrosis, diffuse nephritis, and diffuse splenitis.

The clinical signs and symptoms for Mr. Markov were summarized as: immediate local pain, and general weakness within 5 hours, followed by elevated temperature, nausea, and vomiting 15 to 24 hours later; fever, tachycardia, swollen and sore lymph nodes in the affected groin 36 hours later; hypotension, GI hemorrhage, hypovolemic shock, and renal failure over the next 48 hours; and death on the third day after exposure [50]. A 20-year-old male who injected (s.c.) castor bean extract experienced severe weakness, nausea, dizziness, headache, chest, back, and abdominal pain 36 hours after the injection [79]. He developed a bleeding diathesis, liver failure, and renal failure, and then succumbed to cardiac arrest. A 36-year-old chemist who injected (i.m.) himself with castor seed extract had headache, rigors 10 hours after exposure; developed anorexia, nausea, sinus tachycardia, and lymphadenopathy at the injection sites [80].

There are no documented cases of aerosol exposure to ricin in humans. Lesions induced by oral and parenteral exposure are consistent with those from animal studies, suggesting that the same would hold true for aerosol exposures. An allergic syndrome has been reported in workers exposed to castor bean dust in or around castor oil-processing plants [20]. The clinical picture is characterized by the sudden onset of congestion of the nose and throat, itchiness of the eyes, urticaria, and tightness of the chest.

The effects of oral intoxication vary among individuals, are dose dependent, and have different signs and symptoms. The estimated fatal dose in adults has been reported [81]. Rauber & Heard [82] reviewed 751 cases of castor bean ingestion and reported 14 fatalities (1.9% death rate). The number of beans ingested by patients who died greatly varied. All of the described serious, or fatal cases have the same general clinical history: rapid (less than a few hours) onset of nausea, vomiting, and abdominal pain followed by diarrhea, hemorrhage from the anus, anuria, cramps, dilation of the pupils, fever, thirst, sore throat, headache, vascular collapse, and shock. Death occurred on the third day or later.

The vulnerability of certain populations (e.g., children, pregnant women, the elderly, those with immunosuppression, or underlying respiratory or gastrointestinal tract disease) to the health effects of ricin exposure is unknown; however, persons with pre-existing tissue irritation or damage may sustain further injury upon ricin exposure [83].

Abrin is much more poisonous than ricin [49]. Based on minimal lethal intravenous doses in mice, abrin (0.7 μg/kg) is approximately four times more potent than ricin (2.7 μg/kg; [83]). The human fatal dose has been estimated [40,84]. Predicting the fatal oral dose of jequirity beans is difficult because of lack of data on the bioavailability of abrin [32]. The major symptoms of abrin poisoning are similar to that of ricin. By inhalation, initial symptoms may occur within 8 hours of exposure. After ingestion, symptoms may occur in less than 6 hours but usually are delayed for 1 to 3 days. Death could take place within 36 to 72 hours of exposure, depending on the route of exposure (inhalation, ingestion, or injection) and the dose received. If death has not occurred in 3 to 5 days, the victim usually recovers [49].

Most documented cases of abrin poisoning involve accidental or intentional ingestion of jequirity seeds [35]. Early literature on human poisoning in India was reported by Watt [34]. In the U.S, deaths in children in Florida in 1949, 1958 and 1962 were documented after ingestion of one or more seeds [85]. Two reports in 1955 involved a 37-year-old man who was severely poisoned after ingesting half a seed of *Abrus*, and a 19-year-old girl who died after she was treated for trachoma with jequirity infusions [86]. In Missouri, USA, in 1980, a 6-year-old child who ingested one-half seed whose coat was broken was treated immediately and did not develop any symptoms [87]. A case involving a 15-month-old child who ingested more than 20 jequirity beans was reported to experience only minor features of poisoning, i.e., mild elevation of aspartate aminotransferase, and minor hepatomegaly [88]. The clinical course of a man who ingested a powder made from jequirity beans was characterized (Frohne et al., as cited in [89]). In Sri Lanka in 2001, the management of a 13-year-old boy who ingested two or more jequirity beans was described [90]. A rare case of poisoning involving the white *Abrus* seed variety reportedly caused serious manifestations in a 42-year-old male in Calicut, South India [91]. The patient recovered after a prolonged stay in a hospital without any subsequent complications. A 27-year-old man survived an intentional ingestion of crushed *Abrus* seeds after he was treated with aggressive gastric decontamination and supportive care [92]. Two cases in India detailed unusual manifestations of abrin poisoning (raised intracranial pressure and pappilledema) that have not been described earlier in literature [93]. A recent case report involving a suicidal 20-year-old man demonstrated minimal toxicity despite the ingestion of 10 *Abrus* seeds [94].

Transmission

Ricin and abrin may adhere to skin, nevertheless, ricin or abrin poisoning cannot spread from person to person through casual contact. Ricin is transmitted by the airborne route through release of the toxin in the form of a powder, or a mist, or reaerosolization of ricin into the air from disturbed surfaces [81]. Abrin can also be transmitted through the skin via small pellets or projectiles designed to carry toxin [76].

Diagnosis and Detection

Ricin or abrin poisoning can be diagnosed based on clinical and epidemiological information, e.g., ingestion of seeds, or occurrence of multiple cases during a short period, suggesting a common-source etiology. Diagnosis will rely on the clinician's suspicion in the context of a credible threat or an outbreak of severe gastrointestinal or respiratory illness [95-97].

Two types of laboratory testing are available for suspected ricin exposures. For environmental cases (determined by the CDC for suspected exposures from the environment, or by the FDA for suspected exposures from food or medication), ricin can be detected qualitatively by time-resolved fluorescence immunoassay (TRFIA), and polymerase chain reaction (PCR) in specimens [95,98]. For biological samples, selected specimens can be assessed for urinary ricinine, a marker of ricin exposure, using high performance liquid chromatography electrospray ionization mass spectrometry (HPLC-ESI-MS) [98]. A recent publication described the lateral flow assay (LFA) developed by Miprolab (Göttingen, Germany) as an immunological, sensitive and robust assay to detect toxic amounts of ricin and abrin in food and water [99]. Although detection kits can be obtained from commercial sources, presently, there is no widely available, reliable test that confirms whether a person has been exposed to abrin [49].

Countermeasures

Currently, there is no FDA-approved therapeutic for ricin or abrin exposure. Countermeasures that have demonstrated capability to disrupt the ricin intoxication process include vaccines and antibody therapy. Both rely on the ability of antibody to prevent the binding of ricin to cell receptors, and must be given before exposure to ensure

maximum protection. Treatment for abrin or ricin poisoning is largely symptomatic and basically supportive.

Because of the necrotizing action of these toxins, gastric lavage or induced emesis should be used cautiously. Fluid and electrolyte balance should be monitored and restored if abnormal [76]. Aerosol-exposed patient may require the use of positive-pressure ventilator therapy, fluid and electrolyte replacement, antiinflammatory agents, and analgesics [100]. Dermal exposures require supportive treatment. Percutaneous exposures would necessitate judicious use of intravenous fluids and monitoring for symptoms associated with VLS.

There is currently no vaccine available for abrin or ricin. Development of a ricin vaccine previously focused on either a deglycosylated RTA or formalin-inactivated toxoid [101]. Both vaccines confer protection against aerosolized ricin. Nevertheless, ricin is not completely inactivated by formalin and may retain some of its enzymatic activity (albeit approximately 1,000-fold lower than native ricin). Deglycosylated RTA may lead to local or systemic VLS.

Recent research has focused on developing recombinant RTA subunit vaccines devoid of cytotoxicity and other potential deleterious activities. USAMRIID has engineered a recombinant ricin vaccine 1-33/44-198 (rRTA 1-33/44-198) (RV*Ec*), with increased protein stability over the parent RTA subunit and devoid of enzymatic (N-glycosidase) activity [102], lacking vascular leak activity [103] described in RTA-based immunotoxins, and fully protected vaccinated animals against supralethal aerosol challenges [104,105]. A cGLP pre-clinical toxicity study of RV*Ec* in New Zealand white rabbits demonstrated that no treatment-related or toxicologically significant effects were observed with RV*Ec* during this study [106]. A phase I clinical study is ongoing at USAMRIID to evaluate the safety and immunogenicity of RV*Ec* in humans [107,108].

Rivax, a recombinant protein RTA vaccine, has also been developed [109,110]. Results from the initial Phase I human trial showed that RiVax appeared to be immunological and well tolerated in humans [111]. However, while such results were encouraging, vaccine formulation and stability remain problematic. Hence, a lyophilized formulation that retained immunogenicity when stored at 4°C was developed [112,113].

Animal studies have demonstrated that pretreatment with specific antibodies protected against aerosol exposure to ricin [114,115]. Recent pre-clinical studies also have shown the protection afforded by neutralizing monoclonal antibodies against a lethal dose challenge of ricin [116-118].

Several research groups have engaged in the development of RTA active-site inhibitors or RTB receptor antagonists. Schramm and coworkers [119,120] have synthesized a number of stem-loop RNA analogs that tightly bind to RTA. However, RNA based inhibitors are biochemically labile and have difficulty crossing cell membranes, hence are not expected to be useful anti-ricin drugs [121]. Recently, ricin inhibitors were identified through classical high throughput screening strategies using large libraries of compounds [122]. RTA inhibitors that have been mainly discovered by virtual screening and structure-based design [123-128], including a compound that showed *in vivo* efficacy [129] were also reported. Development of effective small-molecule RIP therapeutics is challenging because strong electrostatic interactions at the RIP·SRL (α-sarcin/ricin loop) interface make these candidate inhibitors ineffective in competing with the rRNA for binding to RIPs [130]. An effective and essentially irreversible RTA inhibitor is thought to be practically useful as a pretreatment for military forces or civilian first-responders [40].

Summary

Ricin and abrin are among the most potent plant toxins known. Ricin was developed as an aerosol biological weapon during WWII, but was never used in battle. As a biological weapon, ricin has not been considered as very powerful in comparison with other agents such as BoNT or anthrax. However, its effectiveness as a discrete weapon of terror-targeted assassinations, biocrimes, or small-scale operations does raise potential concern. Ricin's popularity as well as its track record in actually being exploited by extremists groups and individuals highlight the need to be vigilant of its latent misuse. Abrin has not been used in any wars or terrorist attacks. However, because of its high toxicity, accessibility, and relative ease of preparation, its potential for use as a biological weapon cannot be discounted. The smaller-scale cultivation of *A. precatorius* compared with *R. communis* would seem to suggest a limited, more focused terrorist-type attacks with abrin. Clinical manifestations of ricin and abrin poisoning are very similar, and vary depending on the routes of exposure. Diagnosis is based upon both epidemiological and clinical parameters. Laboratory confirmation of clinical samples is possible by immunoassay but complicated by pharmacokinetic factors. Currently, there is no U.S. FDA-approved drug or vaccine against ricin or abrin poisoning. Treatment is purely supportive and symptomatic. Ricin vaccine candidates are currently in advanced development in laboratory and clinical trials.

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