



Editorial

Forensic Files: Ricin Toxin, A Category B Bioterrorism Agent

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Ricin is a plant derived biological toxin produced by the castor bean plant, *Ricinus communis* L., often referred to as the castor oil plant. The plant itself and the toxin extracted from it are well-known for its toxicity. The plant is widely distributed throughout the world and is native to the Mediterranean, northeast Africa and the Middle East (plant hardiness zones 6-9), growing up to 40ft (12 m) tall in the wild. Global cultivation of the plant, the ease of toxin extraction from castor beans and isolation of the toxin itself, make ricin a very appealing agent, with a strong potential to be employed as a biological weapon [1,2]. The Ricin Toxin (RT) has been known and used as a homicidal poison since antiquity [3]. To date, over seven hundred human intoxications have been reported, dating as far back as the late 1800s [4,5].

Ricin belongs to a family of phytotoxins known as Ribosome Inactivating Proteins (RIPs), specifically type 2 RIPs. RIPs are RNA N-glycosidases that target and deactivate ribosomes required for protein synthesis [6,7]. The holotoxin is comprised of two separate and distinct polypeptide chains, connected by a disulfide bond [8]. The Ricin A chain (RTA) bears the catalytic properties, selectively recognizing and cleaving a distinct adenine from large ribosomal 28s RNA (A4324 for the rat liver ribosome) [9]. This specific purine base is located inside a universally conserved Sarcin/Ricin Loop (SRL) within the large rRNA [10], depurination of which leads to the inhibition of protein synthesis and cellular death. The Ricin B chain (RTB) is divergent and structurally distinct from RTA, with lectin properties (carbohydrate binding domain) [11-13]. The lectin chain is able to bind galactosyl moieties of glycoproteins and/or glycolipids, located on the exterior of eukaryotic cells and promote reverse transport of the RTA to the cytosol [14-16]. The RT accesses translational machinery and depurinates ribosomes after it enters the cytosol. Ricin and other type 2 RIPs owe their toxicity and cytotoxicity to the binding affinity of the B chain to sugar-containing receptors on the cell surfaces. Ricin is an acutely toxic protein, known to induce 50% apoptosis in cells at concentrations below 1 ng/mL.

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Recent reviews provide excellent summaries of ricin trafficking, host cell signal transduction, ribosomal binding and induction of apoptosis [17-21].

The National Institute of Allergy and Infectious Diseases (NIAID) and the Centers for Disease Control (CDC) classifies ricin as a category B priority pathogen [22]. This biotoxin shares the category with such compelling agents as Epsilon toxin (from *Clostridium perfringens*), *Rickettsia prowazekii* (causing typhus fever), Shiga toxin (secreted by diarrheagenic *Escherichia coli* and *Shigella sp.*), *Salmonella sp.* and West Nile virus, among others [22]. Once purified, ricin is a white crystalline, odorless powder, soluble in water and quite stable within a wide pH range [23,24]; it remains stable up to around 80°C [24]. Individual castor beans may have varying ricin content (1 to 5%); ingestion of eight beans is considered lethal [25-27]. Ingestion, inhalation and injection are the primary routes of exposure to the toxin, although absorption through wounded or abraded skin is also possible. Inhalation of ricin leads to diffuse pulmonary edema, increasing the air-blood barrier permeability and alveolar flooding. Initial symptoms of ricin poisoning by inhalation may occur as early as 4 to 8 hours and as late as 24 hours after exposure [28], progressing rapidly, leading to respiratory distress and death due to lymphatic and blood vessel absorption. Ingested toxin readily accumulates in the liver and spleen [29,30]. Following ingestion of ricin, initial symptoms typically occur in less than 10 hours [28]. Injection of ricin presents with a set of nonspecific signs and symptoms similar to sepsis (abdominal pain, nausea, fever, dizziness, headaches and hypotension) and eventually leads to death [26,31-33]. Death from ricin poisoning could take place within 36 to 72 hours of exposure, depending on the route of exposure and the dose received [28]. Being a potent inhibitor of protein synthesis, the effects of ricin on rabbit reticulocyte lysate (IC_{50} 84 nM for native protein and 0.1 nM for reduced toxin) [34] and its toxicity to intact cells (e.g., HeLa cells IC_{50} 0.00067 nM) [35] and animals (e.g., mouse LD_{50} 2.6 µg/kg) [9] are well documented [36]. In previous reports of human castor bean ingestion, the lethal oral dose in humans has been estimated to be 1 to 20 mg of ricin/kg of body weight [26]. The lethal dose of aerosolized RT extrapolated to humans is approximately 350 µg of inhaled toxin for a 70 kg individual [37].

For ages, ricin has been used in assassination attempts. While it is true that ricin and similar toxins are often cited as major bioterrorism threats, it is also understood that they are not very effective with respect to mass dispersal. It should be stressed that in recent years, bioterrorism mass attacks have given way to a "lone wolf" strategy, where the assault is perpetrated by a single individual or small group, decreasing the chances of detection or prevention. In 1952 the US Army filed a patent on how to prepare ricin for weaponry purposes [38]. Ricin was employed in the assassination of Georgi Markov and attempted assassination of Vladimir Kostov, exiled journalists who published incriminating information on the corrupt lives of the Bulgarian communist leadership [3,39,40]. The incidents became legendary not for the poison used - ricin - but rather because of the murder weapon: an umbrella. It is one of the strangest tales of the cold war, peppered with poisons and political intrigue. The "ricin umbrella" delivery system, fired a small pellet filled with ricin, a truly ingenious

mechanism [39-43]. While Markov died three days after the poisoning, Kostov survived; the bullet did not penetrate deep enough to get the toxin into the blood stream, but rather landed in a subcutaneous fat layer thereby saving his life. Several other incidents of attempted usage of ricin are described below. In 1981 Boris Korczak, a CIA double agent, was shot with a ricin-laced pellet in Vienna, VA, penetrating his kidney; there is never been an official investigation pertaining to this case however [44]. In 1982, William A Chanslor a prominent Texas lawyer was found guilty of producing what he believed to be a hard-to-detect "perfect poison" and attempting to euthanize his wife by ricin intoxication [45]. The employment of ricin as a weapon has drastically increased within the past two decades. In the early 1990s, the tax-protesting militia known as the Minnesota Patriot's Council, whose goal was to overthrow the US government, was tried and convicted under the 1989 Biological Weapons Anti-Terrorism Act for possessing ricin [46]. In 1995, an Arkansas man, Thomas Lavy, was charged with possession of 130 g of ricin; the man committed suicide in his jail cell following his arrest [47]. In 1998 (Michigan, US), four members of the North American Militia were arrested and charged with possession of illegal weapons and conspiring to purify ricin [44]. In 2002 (Iraq), the Sunni militant group, Ansar al-Islam, was reported to be testing aerosolized ricin on animals [48]. More recently in 2013, envelopes, addressed to Senator Roger Wicker [49] and President Barack Obama [50], were intercepted and found to be contaminated with ricin. Likewise, in 2013, Shannon Richardson was arrested for sending ricin laced letters to politicians including President Obama and New York City Mayor Michael Bloomberg [51]. Notable examples involving the biological toxin ricin are nicely summarized by Bozza et al., [52].

Several different methods toward ricin detection have been described [52]. These methods aim to detect both biologically active ricin, as well as methods that exploit the intrinsic physical and biochemical properties associated with the toxin. Schramm laboratory has developed a highly sensitive luminescent coupled assay to detect ricin-released adenines from RNA (LOD- limit of detection is 1.6-200 ng/mL) [53]. This method allows for conversion of adenines by adenine phosphoribosyl transferase (APRTase) and Pyruvate orthophosphotase Dikinase (PPDK) to ATP for quantification by firefly luciferase. The resulting AMP is cycled to ATP, producing sustained luminescence proportional to the adenine concentration. Menchior and Tolleson have developed a functional quantitative Polymerase Chain Reaction assay (qPCR) for ricin detection (LOD is 7 ng/mL) [54]. Zhao et al., were able to detect ricin intoxication in mice using serum peptide profiling by MALDI-TOF/MS (LOD is 1 µg/mL) [55]. Several other methods and techniques have been described as well [53].

Throughout the years, various terrorist groups and military organizations have had a great attraction to potent toxins like ricin for weapons, pouring vast resources in their exploration and isolation. To date, there is no prominent therapeutics or vaccines available to either counter or mitigate the effects of ricin (or other deadly RIPs, e.g., shiga toxin) poisoning. In response to growing bioterrorism threats, there is an increasing demand to seek treatments or preventions that counteract ricin poisoning [56,57]. Vaccine prophylactic immunization and post-exposure treatments with therapeutic antibodies have been investigated (e.g., RiVax™, Pulmozyme®, among others); as of yet, none have been approved [37,58-72].

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