

Ricin Toxicity: Clinical and Molecular Aspects

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Abstract

Seeds of the castor bean plant *Ricinus communis* L (CB) contain ricin toxin (RT), one of the most poisonous naturally-occurring substances known. Ricin toxin, a water-soluble glycoprotein that does not partition into the oil extract, is a ribosome-inactivating toxin composed of two chains, labeled A and B. Severity of the toxicity varies depending on the route of exposure to the toxin. Inhalational is the most toxic route, followed by oral ingestion. Orally-ingested RT accumulates in the liver and spleen but other cells are also affected. The main clinical manifestations are also related to the administration route. Oral ingestion of CB or RT results in abdominal pain, vomiting, diarrhea, and various types of gastrointestinal bleeding that leading to volume depletion, hypovolemic shock, and renal failure. Inhalation of the toxin presents with non-cardiogenic pulmonary edema, diffuse necrotizing pneumonia, interstitial and alveolar inflammation, and edema. Local injection of RT induces indurations at the injection site, swelling of regional lymph nodes, hypotension, and death. An enzyme-linked immunosorbent assay (ELISA) has been developed to detect RT in animal tissues and fluids. Ricinine, an alkaloid of CB, can be detected in rat urine within 48 h of RT exposure. Supportive care is the basic treatment and standard biowarfare decontamination protocols are used for RT intoxication. Dexamethasone and difluoromethylornithine might be effective treatments. This review examines the clinical and molecular aspects of ricin toxicity.

Keywords: Biological warfare agent, Castor bean, Ricin, Ricinine

Introduction

Seeds of the castor bean (CB), *Ricinus communis* (*R. communis*), contain ricin, one of the most toxic substances known. Castor bean is wide-spread over the tropical regions (1, 2) and has been cultivated for non-yellowing oil (3). Several ancient civilizations, including those of Greece, Rome, and Egypt used CB oil as an unguent and laxative. Iranians also have used CB for both medical and industrial purposes; as a laxative and oil lamp fuel, respectively. (3). CB oil is now widely used for several medical purposes and for cosmetics, paints, lubricants, and other industrial products (4). Ricin toxin (RT) is a water-soluble glycoprotein that does not partition into the oil extract on phase separation (5, 6).

Peter Hermann Stillmark (1860–1923), the first researcher to extract the toxic glycoprotein from the

CB, named it “ricin.” He also reported that ricin induced hemagglutination and precipitation of serum proteins; however, it is reported that the ricin extracted by Stillmark was a mixture of RT and *R. communis* agglutinin (7). Because of RT’s high toxicity and ease of manufacture, the United State Department of War considered it as a potential weapon in 1918 and named it “W compound.” The British and the US developed a “W bomb” and tested it through World War II; however, it was never used (8).

One of famous case report of assassination by RT was the murder of Georgi Markov, a Bulgarian BBC journalist, by “Umbrella Murder”. He was intoxicated by a RT-infected bullet that was fired from an umbrella into the back of his right thigh (9, 10). In a similar way, Robert Georgia and Porton Down were assassinated

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(5). It is reported that Iraq had weaponized RT in the 1980s (11, 12) and reportedly used it against Iranian soldiers during the Iran-Iraq war (13). Trace amounts of RT have been found in Afghanistan (11).

In 2003, an RT-contaminated letter with no address was found in a Greenville, South Carolina sorting facility (14). More recently, RT-laced letters were also mailed to US President Barack Obama and US Senator Roger Wicker by Shannon Richardson, a Texas actress (15).

Toxicity

Ricin toxin is a potent ribosome-inactivating toxin for mammals; however, different species have different sensitivities; horses are highly sensitive and frogs and chickens are less so (16, 17). The severity of the effects depends on the route of exposure. Inhalation is more potent than oral ingestion (5). The inhalation median lethal dose (LD50) is 3-5 µg/kg while the oral LD50 is 20 mg/kg. This difference may be due to the relatively large molecular size of RT and its degradation through the gastrointestinal (GI) tract. The large molecular size and relatively high charge of RT limit its absorption through intact skin. No dermal toxicity was observed with 50 µg/spot on mice skin tests (17). The RT particle size is an important factor affecting pulmonary deposition and lethality (17).

RT structure and the molecular mechanism of toxicity

Ricin, like cholera and pertussis toxins, belongs to the A–B family of toxins that has two functionally different polypeptide chains, A and B. The A chain, known as ricin toxin A, or RTA, has a molecular weight of 32 kDa, and the B chain, known as ricin toxin B, or RTB, has a molecular weight of 34 kDa (5). The A and B chains are disulfide-linked. Holoricin and RTA have been used in immune-cancer therapy. Ricin toxin A has also been used as a vaccine antigen to induce antibodies (2).

Various RT isoforms with glycosylation differences have been found in different CBs, even in one plant. The amount of RT is related to the bean variety, CB maturity and cultivation conditions; however, no relationship has been found between CB appearance and RT type (5). The best-known RT isoforms include *R. communis* agglutinin (RCA), ricin D (RTD) and ricin E (RTE). *R. communis* agglutinin also binds to red blood cells and induces agglutination and hemolysis. Chromatographic analysis has identified three isoforms of RTD: I, II, and III (18).

Ricin toxin B attaches to terminal galactose residues of cell membrane glycoproteins and it can also bind to mannose. After attachment to the cell membrane, RT enters the cell via endocytosis by clathrin -dependent or -independent pathways; however, most RT is internalized via non-clathrin-coated pit pathways (19). Most of the endocytosed RT molecules are recycled back to the cell surface or degraded in lysosomes. A small minority of the RT molecules transported to endosomes reaches the trans-Golgi network and are then transported to the endoplasmic reticulum (ER). Protein disulfide isomerase of the ER can degrade RTA and RTB by cleaving the disulfide bond. Ricin toxin A inserts into ER membrane and escapes cytosolic proteasomes, which could degrade it.

RTA attaches to 28S ribosomal RNA in the 60S ribosomal subunit and inhibits protein elongation.

One molecule of RTA can inactivate 1500-2000 ribosomes per minute, resulting in cell morbidity (5).

Surface mannose receptors on Kupffer cells, which are macrophages that line the walls of sinusoids in the liver, predispose them to RT toxicity. Administration of an adequate dose of RT can induce injury and result in hepatic failure (17).

Other mechanisms proposed for apoptosis by RT include calcium and magnesium imbalances, release of cytokines, and oxidative stress in the liver (18).

Toxicokinetics

Delipidation and digestion of CBs are essential for the release of RT from the bean matrix. If CBs are swallowed without chewing, the risk of RT intoxication is reduced because of the CB solid shell-like coating. Chewed or crushed seeds and immature CBs are much more toxic than mature intact beans (20, 21). In animal studies, RT gavages induced more severe toxicity than oral administration. This finding may be attributed to carbohydrate structures with terminal galactose residues expressed by GI microbial flora and saccharides that are secreted by salivary or other GI goblet glands. The carbohydrates can compete with GI cell glycolipids and glycoproteins for binding to RT (21).

Ingested RT is absorbed within two hours via blood and lymphatic vessels. The toxin accumulates in the liver and spleen (5). Animal studies showed that RT can be detected in feces within two hours after ingestion, and about 45% of that ingested is excreted unchanged (22). Ricin toxin that is injected intramuscularly or subcutaneously is excreted in urine over 24 hours, with less than 2% fecal excretion (16).

Iodine-125-labeled RT distribution studies in mice found 46% of the input radioactivity in the liver. The spleen and muscles contained 9.9 and 13%; respectively. However, when tissues were compared by weight, the RT was most concentrated in the spleen, which contained 33% of the injected dose per gram of tissue, while the liver and bone marrow contained 7.4 and 5.5%/g, respectively. In 24 hours less than 5% of the injected RT was secreted into the intestine via bile.

Clinical and para-clinical manifestations of animal toxicity

The clinical presentation of RT toxicity depends on the route of administration. Intramuscular injection causes severe localized pain and necrosis of regional lymph nodes and muscles with moderate systemic signs. Inhalation induces respiratory distress with pulmonary and airway lesions. Oral ingestion causes GI hemorrhage and liver, spleen, and kidney necrosis (16, 19, 23). In addition to oral administration routes (gavages and feeding), toxin purity and gastric content volume influence the degree of RT toxicity (21). Intramuscular or subcutaneous injection of RT in sufficient dosage induces necrosis at the injection site, as well as severe local lymphoid necrosis, GI hemorrhage, diffuse nephritis and splenitis, and liver necrosis (16). Intramuscular RT administration of sufficient dosage to mice induces metabolic abnormalities, hypoglycemia, myoglobinuria, renal insufficiency, and increased creatinine kinase, liver transaminase, and amylase (17).

In a rat animal model, intravenous RT injection induced renal, Kupffer, and hepatic cell injuries within four hours and resulted in endothelial cell damage, liver vascular thrombi, hepatocellular necrosis, and disseminated intravascular coagulation (16, 24). Alveolar macrophages, ciliated bronchial cells, and pneumocytes trap aerosolized RT. Pulmonary inflammatory biomarkers such as total protein and the number of broncho-alveolar fluid inflammatory cells increased within one-half day. Permeability of the air-blood barrier increases, which leads to alveolar flooding and subsequent non-cardiogenic pulmonary edema. These events make animals hypoxic and acidotic, leading to respiratory failure and death. Pulmonary histopathological findings in RT-intoxicated animals have shown diffuse necrotizing pneumonia, interstitial and alveolar inflammation, edema, and alveolar flooding (25). Aerosolized RT absorbed through the pulmonary tract induces systemic inflammation secondary to cytokine and chemokine release,

arthralgias, and fever (21); however, a monkey intoxication model showed no systemic absorption (25). Ocular administrations of 1:1,000 to 1:10,000 dilutions of RT solutions induced pseudomembranous conjunctivitis and conjunctival irritation (16).

Ricin toxin is a non-specific cell toxin; nevertheless, the cause of death in RT-intoxicated animals is dependent on the route of exposure (5). Hypotension and vascular collapse secondary to GI necrosis and hemorrhagia with renal and hepatic failure have been reported as the main cause of death in orally-intoxicated animals while pulmonary damage-induced hypoxia might be the cause of death after RT inhalation (5). There is no direct evidence that RT is cardiotoxic or causes arrhythmias (26).

Clinical and paraclinical manifestation of human toxicity

More than 1000 RT poisoning cases secondary to CB consumption have been reported in the literature. Patients present with oropharyngeal irritation, abdominal pain, vomiting, diarrhea 4-6 hours after CB ingestion, and various type of GI bleeding, such as hematemesis, melena, and hematochezia, secondary to GI necrosis (27).

Hypoglycemia and hemolysis are other common manifestations (5). The symptoms may begin five days after exposure, even in previously asymptomatic individuals (21). According to a case report from 2002, a young man subcutaneously injected CB extract and was admitted to the clinic 36 hours later. He suffered from nausea, back and chest pain, headache, severe weakness, dizziness, hematochezia, anuria, and metabolic acidosis. His manifestations progressed to vasoconstrictor-resistant hypotension, and hepatic and renal failures. The bleeding diatheses made the patient resistant to resuscitation after cardiac arrest. On autopsy, the pleura, brain, and myocardium had hemorrhagic foci (28).

Georgi Markov, a Bulgarian journalist, was assassinated by an "Umbrella Murder" injection. He received about 500 µg of RT. He immediately sensed a localized pain at the injection site, and experienced generalized weakness after five hours. On admission, symptoms included fever, nausea, vomiting, tachycardia, and normal blood pressure. The injection site became indurate and regional lymph nodes became swollen. On the second day his heart rate increased to 160 beats per minute, and he became hypotensive and leukocytotic (26,300 cells/mm³). On the next day, his

urine output was reduced. Subsequently, hematemesis and complete atrioventricular conduction block resulted in death (9, 16, 29).

Although there is no evidence of human RT intoxication through inhalation, recent attempts to assassinate two US presidents and a senator by this route have been reported (Ref). Typical allergic rhinitis, including urticaria, throat and nose congestion, itching eyes, and chest tightness have been reported by workers exposed to CB dust (30). There are case reports of suicide attempts using self-made CB seed extracts through intravenous, intramuscular, and subcutaneous injections, and oral ingestion (4).

Detection testing and diagnosis

The diagnosis of RT-mediated pulmonary toxicity is usually established based on clinical manifestations and presentations. Pulmonary distress, GI hemorrhage, hypotension, rapid onset of the vascular leak syndromes (VLS), and edema make ricin suitable for use as a biological warfare agent (BWA) (5).

As mentioned above, RT intoxication lacks specific manifestations (5). Clinical symptoms vary from mild to aggressive, as with intoxication by similar toxins, such as abrin, and common pulmonary diseases, such as exacerbation of chronic obstructive pulmonary disease or asthma. In addition, clinical manifestations of oral RT intoxication are dose-dependent, and during the first six hours after ingestion, different types of infectious gastroenteritis are on differential diagnoses lists (16).

Ricin toxin in animal tissues and fluids can be detected by enzyme-linked immunosorbent assays (ELISA). This method is also applied in human specimens with a lower limit of detection of 0.1 ng/mL (1.54 pmol/L) (20). Ricinine, an alkaloid of CB, can be detected in rat urine for 48 h after RT exposure (31). Ricin-antibody conjugates can also be detected in surviving patients two weeks after ingestion (20, 32).

Various methods for the detection of RT in body fluids, tissues, food, water, and materials or equipment that have been in contact with ricin have been suggested, but no standard and approved RT detection method currently exists.

Medical management

The rapid and irreversible action of RT makes effective treatment of intoxicated patients difficult; therefore, high risk groups such as military and diplomatic personnel

should be vaccinated (Ref). Decontamination protocols for BWA are also used for RT intoxication cases. Supportive care is the basic treatment for ricin intoxication, which depends on the route of exposure (Ref). Correction of fluid and electrolyte imbalances and monitoring of liver and renal functions are the first treatment steps. The coagulopathies should be corrected as well. In ricin poisoning by inhalation, respiratory care such as administration of anti-inflammatory drugs, analgesics, positive-pressure breathing, and artificial ventilation are also indicated (16, 33-35).

Dexamethasone and difluoromethylornithine were more effective than the antioxidants butylatedhydroxyanisole and vitamin E succinate at extending survival times in intoxicated mice (36). Anti-RTA, anti-RTB, and anti-RT antibodies are able to protect cells against RT by preventing binding, internalization, or routing of RTA to the endosomal compartment, changing intracellular trafficking, and neutralizing the ricin inside the cell. Anti-RT antibody protected animals 8-12 hours after ricin exposure (32, 37)

Conclusion

Ricin is a potent cell toxin from the bean of the castor plant *R. communis*, which has been used as a biological warfare and assassination agent because of its ease of access, relative ease of extraction, and stability. Toxicity results from protein synthesis inhibition, but other mechanisms should be noted including apoptosis pathways, magnesium and calcium imbalances, cytokine release, acute phase reactions, and oxidative stress in the liver. Use of ricin as a terrorism weapon highlights the need for clinicians and public health officials to be aware of ricin-associated illness. Diagnosis of RT intoxication may be difficult, especially on the first encounter, and ricin poisoning manifestations vary depending on the exposure route. Oral ingestion of RT causes GI hemorrhage and liver, spleen, and kidney necrosis. Intramuscular injection induces severe localized pain and necrosis of regional lymph nodes and muscles with moderate systemic signs. Inhalation causes respiratory distress with pulmonary and airway lesions. Currently various techniques exist for the identification of RT in tissue sections, body fluids, environmental samples, and food. Treatment of RT intoxicated patients is difficult because the toxin acts rapidly and

irreversibly; therefore, vaccination of high risk groups as a preventative measure is an important defensive strategy. Because of its cytotoxic effects, modified ricin offers promise as a therapeutic agent for the selective killing of unwanted cells, such as in cancer therapy.

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