Chapter 15

RICIN

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INTRODUCTION

Ricin is a protein isolated from the seeds of the castor bean plant (*Ricinus communis*). Like abrin (from the seeds of the rosary pea, *Abrus precatorius*), ricin is a lectin and a member of a group of ribosome-inactivating proteins that block protein synthesis in eukaryotic ribosomes.[1]

The castor bean is native to Africa, but it has been introduced and cultivated throughout the tropical and subtropical world. Although tolerant to a wide temperature range, it grows best in elevated year-round temperatures and rapidly succumbs to subfreezing temperatures. However, it is often grown as an ornamental annual in temperate zones. The seeds are commercially cultivated in many regions of the world, predominantly in Brazil, Ecuador, Ethiopia, Haiti, India, and Thailand. The beans contain 35% to 55% by weight of fast-drying, nonyellowing oil used in the manufacture of lubricants, inks, varnishes, and dyes. After oil extraction, the remaining seed cake may be detoxified by heat treatment and used as an animal feed supplement. The seed hulls are similar to barnyard manure in their fertilizer value.

The toxicity of castor beans has been known since ancient times, and more than 750 cases of intoxication in humans have been described.[2] Although considerably less potent than botulinum neurotoxins and staphylococcal enterotoxins, ricin represents a significant potential biological weapon because of its stability and worldwide availability as a by-product of castor oil production. In addition, it has been associated with several terrorist actions and therefore may be a potential agent of bioterror.

HISTORY

R communis was cultivated for centuries in ancient Egypt and Greece for the lubricating and laxative effects of its oil. In addition, both the oil and whole seeds have been used in various parts of the world for disease treatment as well as for malicious mischief and homicidal purposes.[3] During World War I, the excellent lubricating properties of castor oil were utilized by the wartime aircraft industry. Shortages of castor oil during World War II resulted in US government subsidies for agricultural production of castor beans in the San Joaquin Valley of California. These subsidies persisted until the 1960s, when synthetic oils replaced castor oil in the aircraft industry. There is no commercial production of castor oil in the United States today.

The first toxinology work on ricin was performed by Hermann Stillmark at the Dorpat University in Estonia for his 1888 thesis.[4] Stillmark determined that ricin was a protein and suggested the name. He purified ricin to a very high degree (although not completely to homogeneity) and found that it agglutinated erythrocytes and precipitated serum proteins.[5] For years, these effects were considered to be the mechanism of action of ricin, although later work showed that the toxicity and agglutination effects were separable properties.

In 1891 Paul Ehrlich studied ricin and abrin in pioneering research that is now recognized as the foundation of immunology.[5] Following the lead of Indian farmers who had known for centuries that calves could be protected from abrin poisoning by feeding them small amounts of *Abrus* seeds, Ehrlich vaccinated animals with small oral doses of castor beans. After protection was established, he continued vaccinating with subcutaneous injections of toxin. Experiments with the serum of immune animals led him to discover that the immunity was specific, was associated with serum proteins, and could be transferred to the offspring through milk.

At the end of the 19th century, with the rising interest in bacterial toxins, interest in plant toxins waned. It wasn't until the mid-20th century, with the discovery that ricin inhibited protein synthesis and thus might be useful for treating cancer, that the scientific community "rediscovered" ricin. Olsnes and Pihl[6] demonstrated that protein synthesis was strongly inhibited in a cell-free rabbit reticulocyte system, and suggested that the effects resulted from inhibited elongation of the nascent polypeptide chain. They also determined that ricin consisted of two dissimilar polypeptide subunits and that the A chain was responsible for the toxic action. Results from this laboratory over the next few years revealed the 60S ribosomal subunit as the enzymatic target and led to further characterization of the enzymatic action.[7]

More recently, the inhibitory action of ricin on protein synthesis in eukaryotic cells was investigated as a potential chemotherapeutic agent against some forms of cancer. The active subunit of ricin is specifically targeted to tumor cells by conjugation to tumor-specific antibodies. These chimeric toxins, called immunotoxins, have been tested against several forms of cancer, with promising results.[8] However, side effects such as nonspecific hepatic toxicity and vascular leak syndrome (VLS) have been problematic and dose limiting. Recent work by Smallshaw and coworkers[9] has demonstrated that the VLS activity of the toxin is mediated by a discrete sequence moiety separate

from the region related to protein synthesis inhibition. Specifically, mutations in a three-amino acid motif of the ricin A chain yielded an immunotoxin with significantly reduced VLS side effects with no loss of cytotoxicity. Testing in a mouse model demonstrated improved effectiveness, suggesting that ricin immunotoxins may yet have a place in the anticancer armamentarium.

Because of its potency, worldwide availability, and ease of production, the US Chemical Warfare Service began considering ricin as a potential biological warfare agent near the end of World War I. The research involved methods of adhering ricin to shrapnel and the production of effective aerosol clouds.[10] However, the war ended before the evolution of weaponry based upon this research. During World War II, the Americans and British collaborated on the development of a ricin-containing bomb (the so-called "W bomb"). Although they were tested, these bombs were never used in battle. The United States unilaterally ended its offensive biological warfare program in 1969–1970; all offensive research and development were terminated, and remaining stocks of ricin munitions were destroyed in 1971–1972. The 1975 Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction prohibited the development, production, and storage of any toxin for offensive purposes.

In addition to its coverage under the 1975 convention, ricin and one other toxin (saxitoxin) were also specifically included under the 1993 Chemical Weapons Convention, ratified by Congress in 1997. In the United States, ricin and abrin are both included in the Centers for Disease Control and Prevention's select agent list of toxins requiring certification for possession and transfer. The US intelligence community believes that ricin was included in the biological warfare programs of the Soviet Union, Iraq, and possibly other nations as well.

In recent years, ricin has drawn the interest of extremist groups. Such notoriety is likely driven by the ready availability of castor beans, ease of toxin extraction, coverage in the popular press, and popularization on the Internet. Several individuals have been arrested under the 1989 Biological Weapons Anti-Terrorism Act for possessing ricin. In the past few years alone, various major news organizations have reported the following stories:

- 2002: Ricin was discovered in the apartment of six terrorist suspects arrested in Manchester, England.
- 2003: An envelope containing a sealed container of ricin and a note threatening to contaminate water supplies was processed at a mail facility in Greenville, South Carolina.
- 2004: Traces of ricin were discovered in the mail room of the Dirksen Senate Office Building in Washington, DC.

While none of these events resulted in any known human intoxications, they clearly demonstrate that ricin is well known, available to and recognized by extremist groups, and should be seriously considered as a potential bioterrorist threat agent.

DESCRIPTION OF THE AGENT

Ricin is a 66-kd globular protein that typically makes up 1% to 5% of the dry weight of the castor bean, although the yield can be highly variable.[11] The toxic form is a heterodimer consisting of a 32-kd A chain connected to the 32-kd B chain through a single disulfide bond.[12] As such, it is a member of the type II family of ribosome-inactivating proteins (RIPs), which possess enhanced in-vivo toxicity because of the presence of the B chain that facilitates uptake by the cell. Type I RIPs lack the B chain, and cellular toxicity is much less; uptake depends on endocytosis. Both chains are glycoproteins containing multiple mannose residues on their surfaces; association of both chains is required for toxicity.

Purification and characterization is not difficult, and the crystal structure has been determined to .25 nm.[13] Each chain is a globular protein, with the A chain tucked into a gap between two roughly spherical domains of the B chain. A lactose disaccharide moiety is bound to each of these spherical domains. The disulfide bond links residue 259 of the A chain with residue 4 of the B chain. The crystal structure demonstrates a putative active cleft in the A chain, which is believed to be the site of enzymatic action. A functional lipase active site at the interface of the two subunits was recently identified.[14] This site is thought to be important for intracellular A chain translocation and subsequent intracellular trafficking (see below). Recombinant A and B chains, as well as specific mutants, have been expressed and characterized in several expression systems including *Escherichia coli*.[15-18]

Toxicity

Ricin is recognized as one of the most exquisitely toxic plant-derived RIPs identified to date.[19] However, considerable variation in potency exists among species.

For instance, on a mg/kg basis, potency varies over two orders of magnitude between species of domestic and laboratory animals; chickens and frogs are the least sensitive, and horses are the most sensitive.[20] Potency also varies greatly with route of administration. In laboratory mice, approximate median lethal dose values and time to death are, respectively, 5 μg/kg and 90 hours by intravenous injection, 22 μg/kg and 100 hours by intraperitoneal injection, and 24 μg/kg and 100 hours by subcutaneous injection. Ricin is extremely toxic by inhalation; median lethal dose estimates range from 3 to 15 μg/kg in rodents and primates (Table 15-1). In contrast, ricin is least potent by the oral route; median lethal dose estimates in mice are approximately 20 mg/kg. Low potency by the oral route likely reflects poor absorption and possibly partial degradation in the gut. Higher potency by other routes may be related to the ubiquitous nature of toxin receptors among cell types. In skin tests on mice, no dermal toxicity was observed at 50 μg/spot, suggesting poor dermal absorption of this large, highly charged protein.[21]

Pathogenesis

The mechanism of action of ricin is similar to that of other type II RIPs. The two-chain structure is key to cellular internalization and subsequent toxicity. The lectin properties of the B chain enable toxin binding to cell-surface carbohydrates, and the A chain possesses the enzymatic activity. Initial binding of the B chain to glycoside residues on glycoproteins and glycolip-

TABLE 15-1

MEDIAN LETHAL DOSES FOR AEROSOLIZED RICIN IN VARIOUS ANIMAL SPECIES

Species	Strain	LD_{50} (μg/kg)
Mouse (*Mus musculus*)	BALB/c	11.2
	BXSB	2.8
	Swiss Webster	4.9
	CBA/J, C57/BL/6J, L2H/HeJ	5.3
	A/J	8.2
	C3H/HeN	9.0
Rat (*Rattus norvegicus*)	Fisher 344	5.3
African green monkey (*Chlorocebus aethiops*)		5.8
Rhesus monkey (*Macaca mulatta*)		15.0

LD_{50}: medial lethal dose

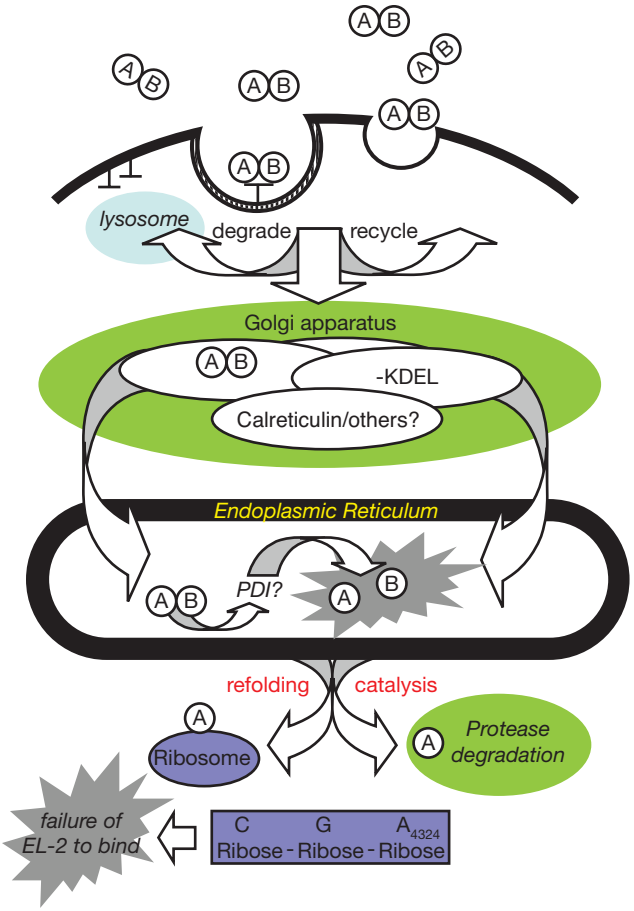

Fig. 15-1. Binding, internalization, and intracellular tracking of ricin leading to enzymatic action at the 60S ribosome. Endosomes transport ricin from the initial binding site to the Golgi apparatus (and may also traffic the internalized ricin back to the cell surface or to lysosomal degradation). Then, calreticulin and possibly other proteins are thought to chaperone the ricin from the Golgi apparatus to the endoplasmic reticulum (ER). At the ER, protein disulfide isomerase may reduce the disulfide bridge between the ricin subunits, facilitating unfolding and retrograde transport of the A chain through the ER lumen via a Sec61-mediated translocon. In the cytoplasm, the A chain can interact with the ribosome, which acts as a suicidal chaperone stimulating proper refolding and resumption of catalytic activity. The A chain cleaves one specific adenosine residue (A4324) near the 3′ end of 28S ribosomal RNA, which blocks elongation factor-2 binding, thus inhibiting protein synthesis.
A: ricin A chain
A4324: adenosine residue 4324
B: ricin B chain
EL-2: elongation factor 2
-KDEL: amino acid sequence at the C-terminal of a soluble protein in the lumen of a membrane or a C-terminal Lys-Asp-Glu-Leu sequence
PDI: protein disulfide isomerase
Illustration: Courtesy of Chad Roy, Tulane National Primate Research Center, Covington, Louisiana.

ids triggers endocytic uptake of the toxin. Increased binding is observed in cell types rich in mannose receptors; dissociation of ricin from its binding sites is increased in the presence of lactose.[22] There are a number of possible endocytic mechanisms for cell entry, some of which are independent of cell coat-binding protein (clathrin) action.[23] Trafficking of the toxin within the cell from the initial binding site to the Golgi apparatus occurs via endosomal transport and is seemingly regulated by intracellular calcium.[24] Early endosomes may also traffic the internalized ricin back to the cell surface or to lysosomal degradation (Figure 15-1). A Golgi-associated type II-α protein kinase also largely regulates toxin transport in specific cell types such as lymphocytes.[25] Association with the Golgi apparatus seems to be a requirement for further trafficking to the endoplasmic reticulum (ER).[26] Transport from the Golgi apparatus to the ER is thought to be in association with one or more chaperone proteins, most notably calreticulin.[27] Once delivered to the ER, protein disulfide isomerase may reduce the disulfide bridge between the subunits, facilitating unfolding and retrograde transport of the A chain through the ER lumen via a Sec61-mediated translocon.[28] ER processing and transport to the cytosol is a critical step; only when the holotoxin is reduced by novel chaperones such as protein disulfide isomerase can subsequent ribosomal inactivation take place in the cytosol. As with related toxins, transport to the cytosol is the rate-limiting step during the decline in protein synthesis.[29] Once transported from the ER to the cytoplasm, the A chain can interact with the ribosome, which acts as a suicidal chaperone stimulating proper refolding and resumption of catalytic activity.[28] The Michaelis constant for enzymatic action at the ribosome is 0.1 μmol/L and the enzymatic constant is 1,500/min. It cleaves one specific adenosine residue (A4324) near the 3′ end of 28S ribosomal RNA. This targeted cleavage blocks elongation factor-2 binding, thus inhibiting protein synthesis.[30] The rate of ribosomal inactivation easily overwhelms repair mechanisms and kills the cell.

CLINICAL SYMPTOMS, SIGNS, AND PATHOLOGY

Animal studies indicate that clinical signs and pathological changes in ricin intoxication are largely route specific. Ingestion causes gastrointestinal symptoms including hemorrhage and necrosis of liver, spleen, and kidneys; intramuscular intoxication causes severe localized pain, muscle and regional lymph node necrosis, and moderate systemic symptoms; inhalation results in respiratory distress with airway and pulmonary lesions. Transient leukocytosis appears to be a constant feature in humans, whether intoxication is by injection or oral ingestion. Leukocyte counts 2- to 5-fold above normal are characteristic findings among cancer patients receiving ricin immunotoxin therapy, and also in the case of the Bulgarian dissident Georgi Markov during his agonizing death after a successful assassination attempt.[31]

Oral Intoxication

Ricin is less toxic by oral ingestion than by other routes, probably due to poor absorption of the toxin and possibly partial enzymatic degradation in the digestive tract. In animal models, a significant amount of orally administered ricin is found in the large intestine 24 hours postingestion with limited systemic uptake.[32] Most cases of oral ingestion are related to ingestion of castor beans, and the severity of intoxication varies with the degree of mastication of the beans. Review of the literature reveals mostly nonfatal case reports of castor bean ingestion in the United States and a few fatal case reports from abroad. A review of the American Association of Poison Control Center's Toxic Exposure Surveillance System from 1983 to 2002 notes no reported fatalities from ricin poisoning.[33]

A recent review article[34] summarizes symptoms of substantial castor bean ingestion. The authors note oropharyngeal irritation, vomiting, abdominal pain, and diarrhea beginning within a few hours of ingestion. Local necrosis in the gastrointestinal tract may lead to hematemesis, hematochezia, and/or melena. The resultant loss of fluid and electrolytes may lead to hypotension, tachycardia, dehydration, and cyanosis. Significant fluid loss may lead to renal failure and hypovolemic shock. A portion of the toxin is absorbed through the gastrointestinal tract leading to systemic signs. In oral (and parenteral) intoxication, cells in the reticuloendothelial system, such as Kupffer cells and macrophages, are particularly susceptible, due to the mannose receptor present in macrophages.[35] The effect on these cells may lead to liver damage, which may persist for several days and may progress to liver failure at higher doses.

In 1985 Rauber and Heard[2] summarized the findings from their study of 751 cases of castor bean ingestion. There were 14 fatalities in this study, constituting a death rate of 1.9%—much lower than traditionally believed. Twelve of the 14 cases resulting in death occurred before 1930. Even with little or no effective supportive care, the death rate in symptomatic patients has been low—in the range of 6%. The reported number

of beans ingested by patients who died varied greatly. Of the two lethal cases involving oral intoxication documented since 1930, one involved a 24-year-old man who ate 15 to 20 beans, and the other involved a 15-year-old boy who ate 10 to 12 beans. All of the reported serious, or fatal, cases of castor bean ingestion 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 3rd day or later. The most common autopsy findings in oral intoxication were multifocal ulcerations and hemorrhages of gastric and small-intestinal mucosa. Lymphoid necrosis in the mesenteric lymph nodes, gut-associated lymphoid tissue, and spleen were also present, as were Kupffer cell and liver necrosis, diffuse nephritis, and diffuse splenitis.

Injection

Intramuscular or subcutaneous injection of high doses of ricin in humans results in severe local lymphoid necrosis, gastrointestinal hemorrhage, liver necrosis, diffuse nephritis, and diffuse splenitis. Injection of ricin leads to necrosis at the injection site, which may predispose one to secondary infection.[36] A case report of a 20-year-old male who injected castor bean extract to commit suicide describes in detail the clinical course. This patient was admitted 36 hours after the injection with severe weakness, nausea, dizziness, headache, and pain in the chest, abdomen, and back. Examination revealed hypotension, anuria, metabolic acidosis, and hematochezia. He subsequently developed a bleeding diathesis, liver failure, and renal failure. Despite maximal treatment with vasopressors and treatment of the bleeding diathesis, he developed cardiac arrest and was unable to be resuscitated. Postmortem examination revealed hemorrhagic foci in the brain, myocardium, and the pleura.[37]

In the case of Georgi Markov,[31] the lethal injected dose was estimated to be as much as 500 μg. This resulted in almost immediate local pain, followed by general weakness within about 5 hours. Fifteen to 24 hours later, he exhibited elevated temperature, nausea, and vomiting. Thirty-six hours after the incident, he was admitted to the hospital feeling ill and exhibiting fever and tachycardia. Blood pressure was normal. Lymph nodes in the affected groin were swollen and sore, and a 6-cm diameter area of induration was observed at the injection site on his thigh. Just over 2 days after the attack, he suddenly became hypotensive and tachycardic. His pulse rate increased to 160 beats per minute, and white blood count rose to 26,300/mm^3. Early on the third day, he became anuric and began vomiting blood. An electrocardiogram demonstrated complete atrioventricular conduction block. Markov died shortly thereafter. At the time of death, his white blood count was 33,200/mm^3. A mild pulmonary edema was thought to have been secondary to cardiac failure.

Inhalation

Although data on aerosol exposure to ricin in humans are not available, lesions induced by oral and parenteral exposure are consistent with those from animal studies, suggesting that the same would hold true for aerosol exposures. In humans, an allergic syndrome has been reported in workers exposed to castor bean dust in or around castor oil-processing plants.[38] 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. In more severe cases, wheezing can last for several hours, and may lead to bronchial asthma. Affected individuals respond to symptomatic therapy and removal from the exposure source. These patients may have had castor bean-positive skin prick tests, possess specific IgE against castor beans by the radioallergosorbent test technique, and may also have responded to a nasal challenge test with castor bean pollen.[39] It is likely, however, that these responses occurred as a result of exposure to bean constituents other than ricin.

Studies in mice demonstrate that aerosolized ricin is deposited in the trachea and lungs. This is followed by a decrease in detectable ricin in the lung and an increase in the trachea, likely due to pulmonary clearance via the mucociliary escalator. Pulmonary deposition is highly dependent upon aerosol particle size, which profoundly affects lethality in this animal model.[40] Immunohistochemistry studies in rats exposed to ricin by aerosol indicate that aerosolized ricin binds to ciliated bronchial cells, alveolar macrophages, and alveolar lining cells[41] (Figure 15-2). Inhalational exposure of rats results in a diffuse necrotizing pneumonia of the airways, with interstitial and alveolar inflammation as well as edema.[42] No notable changes in lung injury parameters occur before 8 hours postchallenge. By 12 hours, inflammatory cell counts and total protein (both from fluid obtained via bronchoalveolar lavage) increase, suggesting both enhanced permeability of the air–blood barrier and cytotoxicity. These findings are associated with a blood-cell analysis indicating inflammation. By 18 hours postchallenge, alveolar flooding is present, and extravascular lung water is increased. Both continue to increase for up to 30 hours. At 30 hours postchallenge, arterial hypoxemia and acidosis

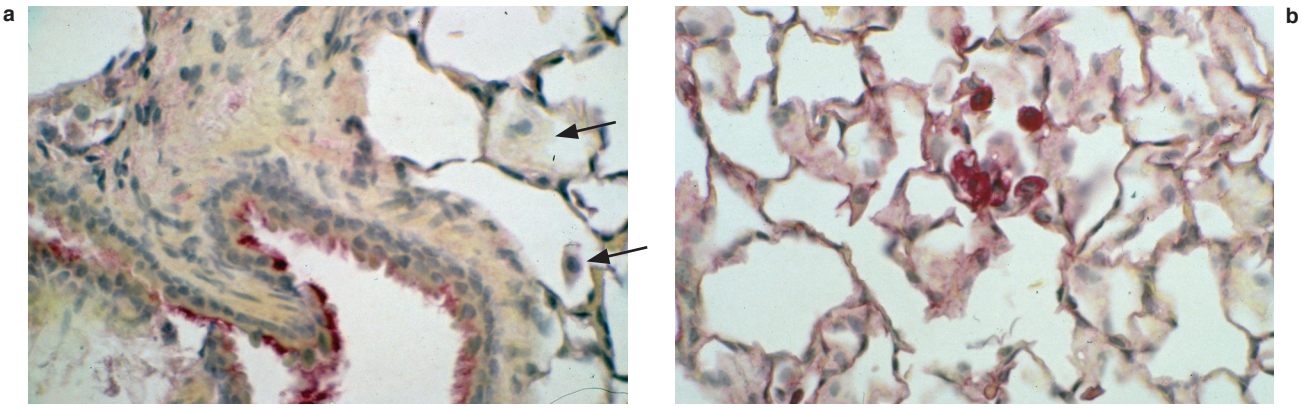

Fig. 15-2. Lung from a rat exposed to ricin by aerosol. Immunocytochemical stain for ricin demonstrates strong reactivity for (**a**) airway epithelial cells and alveolar macrophages (arrows) and (**b**) alveolar lining cells (immunocytochemical stain, original magnification x 50). Photographs: Courtesy of Lieutenant Colonel CL Wilhelmsen, DVM, PhD, Veterinary Corps, US Army, Division of Pathology, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

are present, and histopathological evidence of alveolar flooding becomes significant.

Inhalation toxicity in nonhuman primates is characterized by a dose-dependent preclinical period of 8 to 24 hours, followed by anorexia and progressive decrease in physical activity. Death occurs 36 to 48 hours postchallenge and is dose-dependent. Relevant gross and histopathological changes are confined to the thoracic cavity (Figure 15-3). All monkeys in this study developed acute marked-to-severe fibrinopurulent

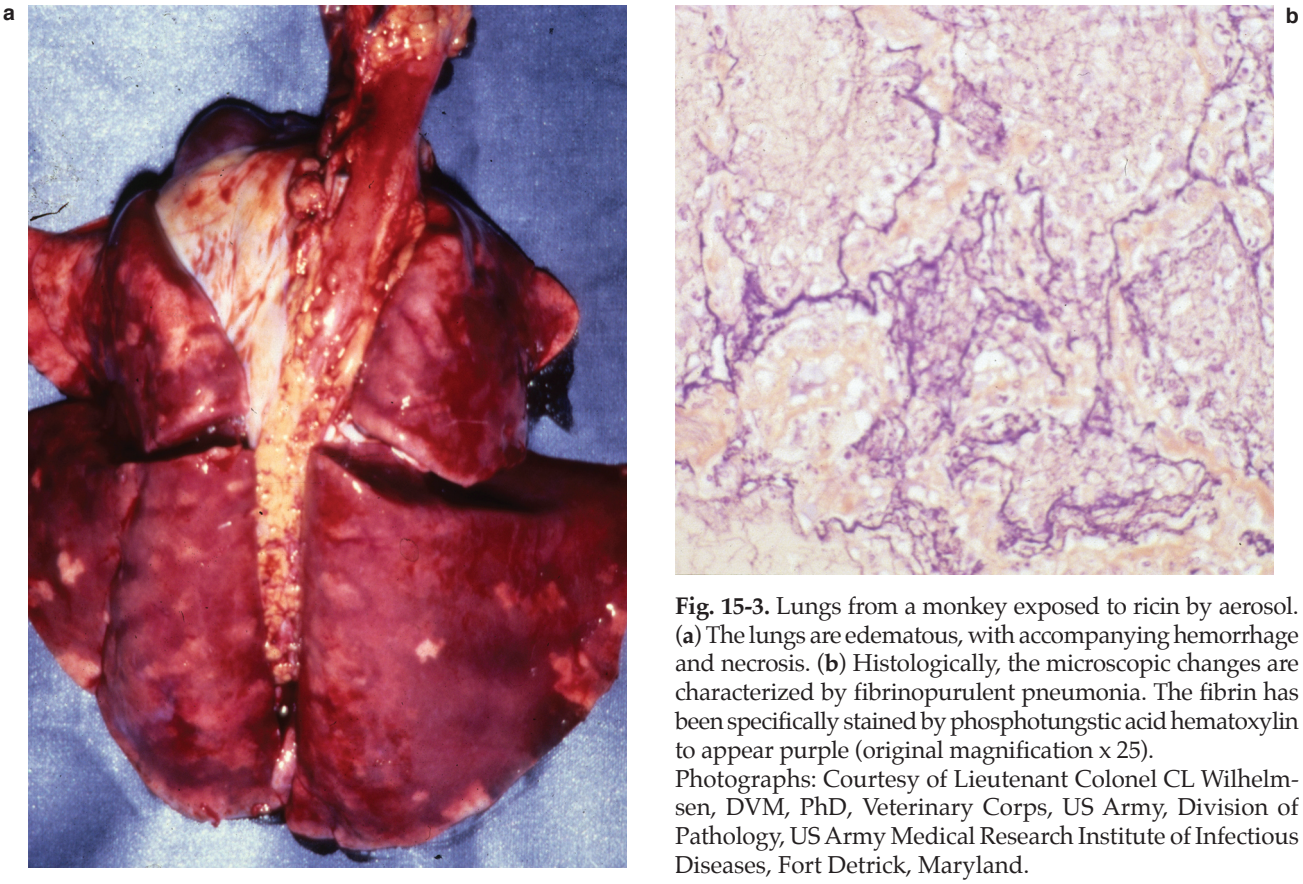

Fig. 15-3. Lungs from a monkey exposed to ricin by aerosol. (**a**) The lungs are edematous, with accompanying hemorrhage and necrosis. (**b**) Histologically, the microscopic changes are characterized by fibrinopurulent pneumonia. The fibrin has been specifically stained by phosphotungstic acid hematoxylin to appear purple (original magnification x 25).
Photographs: Courtesy of Lieutenant Colonel CL Wilhelmsen, DVM, PhD, Veterinary Corps, US Army, Division of Pathology, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

pneumonia, with variable degrees of diffuse necrosis and acute inflammation of airways. There were also diffuse, severe alveolar flooding and peribronchovascular edema (Figure 15-4), acute tracheitis, and marked-to-severe purulent mediastinal lymphadenitis. Two monkeys had acute adrenalitis.[43]

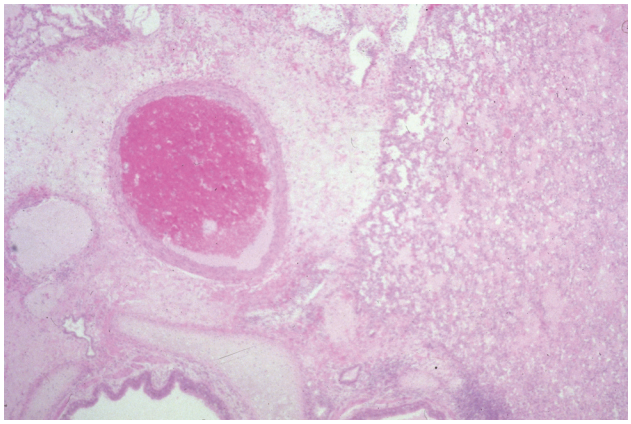

Fig. 15-4. Widespread perivascular and peribronchiolar edema in a monkey, a characteristic finding in aerosol ricin intoxication (hematoxylin-eosin stain, original magnification x 10).
Photograph: Courtesy of CL Wilhelmsen, DVM, PhD, Lieutenant Colonel, Veterinary Corps, US Army, Division of Pathology, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

Cause of Death

The exact cause of death is unknown and probably varies with route of intoxication. Ingesting the toxin results in ulceration and hemorrhage of the stomach and small intestine mucosa, necrosis of the mesenteric lymphatics, liver necrosis, nephritis, and splenitis. Resultant loss of fluid and electrolytes may lead to hypotension, tachycardia, dehydration, cyanosis, and vascular collapse. Injection of the toxin may lead to severe local lymphoid necrosis, gastrointestinal hemorrhage, liver necrosis, diffuse nephritis, and diffuse splenitis. High doses administered intravenously in laboratory animals are associated with disseminated intravascular coagulation, and it has been suggested that hepatocellular and renal lesions result from vascular disturbances induced by the toxin rather than a direct effect of the toxin itself.[44] Early studies[45,46] clearly established that intravenous administration of ricin to rats results in diffuse damage to Kupffer cells within 4 hours, followed by endothelial cell damage, formation of thrombi in the liver vasculature, and finally, hepatocellular necrosis. In mice, rats, and primates, high doses by inhalation apparently produce lethal pulmonary damage, probably due to hypoxemia resulting from massive pulmonary edema and alveolar flooding.

DIAGNOSIS

As with other potential intoxications on the unconventional battlefield, epidemiological findings will likely play a central role in diagnosis. The observation of multiple cases involving severe pulmonary distress in a population of previously healthy young soldiers, linked with a history of being at the same place and time during climatic conditions suitable for a biological warfare attack, would suggest an aerosol exposure. Additionally, ingestion should be suspected in the case of several soldiers with gastrointestinal hemorrhage and hypotension who have eaten from the same food source. In patients who may be targets of an assassination attempt, ricin injection should be considered if there are signs of rapid onset of symptoms similar to VLS.

The differential diagnosis of aerosol exposure to ricin should include staphylococcal enterotoxin B and exposure to pyrolysis by-products of organofluorine polymers, such as Teflon and Kevlar (both manufactured by DuPont, Wilmington, Del), or other organohalides, oxides of nitrogen, and phosgene. Insecticides, although not expected in a battlefield scenario, can be spread aerially over large geographical areas and should be included in the differential diagnosis. The differential diagnosis of ingested ricin includes enteric pathogens, enterotoxins, and other toxins, including caustic agents, mushroom species, hydrocarbons, and pharmaceuticals such as salicylates and colchicine.

After inhalational intoxication in laboratory animals, findings are generally nonspecific. Confirmation of inhalational intoxication in humans would most likely be through immunological analysis of a swab sample from the skin or nasal mucosa. Ricin can be identified from such samples by immunoassay for at least 24 hours postexposure.[47] Because ricin is extremely immunogenic, individuals surviving a ricin attack would likely have circulating antibody within 2 weeks of exposure. Therefore, serum samples should be obtained from survivors. Immunoassay of blood or other body fluids may be useful for confirming ricin intoxication. This test can accurately measure ricin to less than 1 ng/mL in clinical matrices.[48] However, because ricin is bound very quickly regardless of exposure route and metabolized before excretion,[49] identification in body

fluids or tissues is difficult. Although analytical methods for detecting the toxin are available from reference labs, including the US Army Medical Research Institute of Infectious Diseases and the Centers for Disease Control and Prevention there are no clinically validated methods to detect ricin in biological fluids.[50] Postmortem identification of toxin in tissues can be accomplished through immunohistochemistry.

MEDICAL MANAGEMENT

The potential scenarios in which ricin intoxication might be seen by military medical personnel are: (*a*) small-scale battlefield or terrorist delivery of an aerosol; (*b*) parenteral administration of the toxin to an individual by an assassin's tool; or (*c*) contamination of food sources. Because ricin acts rapidly and irreversibly (directly on lung parenchyma after inhalation or distributed quickly to vital organs after parenteral exposure), postexposure therapy is difficult. Therefore, vaccinating personnel at risk for ricin exposure is an important consideration.

Vaccination and Passive Protection

Inhalational exposure is best countered with active vaccination. However, there is currently no licensed vaccine available. Development of a ricin vaccine has previously focused on either a deglycosylated ricin A chain or formalin-inactivated toxoid.[51] Both preparations confer protection against aerosolized ricin. However, 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 ricin A chain may lead to local or systemic VLS.

More recent research has evaluated recombinant ricin A chains to eliminate toxicity and improve the stability of the vaccine. An optimized vaccine candidate, RTA 1-33/44-198, was developed by the US Army by structurally modifying the ribosome-inactivating protein fold to create a nonfunctional scaffold for presentation of a specific protective epitope. This vaccine candidate protected 100% of vaccinated animals against supralethal aerosol challenges.[52] Other mutants of recombinant ricin A chains devoid of enzymatic activity have also been developed. However, these mutants may still induce VLS in vaccinated individuals. A vaccine candidate based on a mutation of both the enzymatic site and the VLS-inducing site has been developed by a research group in Texas.[53] This candidate, RiVax (DOR BioPharma, Inc, Miami, Fla) is at least 10,000-fold less active than wild-type ricin A chain regarding inhibition of protein synthesis, and does not induce VLS. RiVax also protected mice against intraperitoneal challenge of up to 10 median lethal doses. The producer is now beginning phase I clinical trials to assess the safety and immunogenicity of RiVax in humans.

Passive protection with aerosolized anti-ricin immunoglobulin (IgG) has also been evaluated as prophylaxis before aerosol challenge. Administration of nebulized anti-ricin IgG effectively protected against lung lesions and lethality in mice when challenged with an aerosol exposure to ricin approximately 1 hour later. Extrapolation of these data to clearance rates of IgG from the airways of rabbits suggests that anti-ricin–specific antibodies may provide protection for up to 2 to 3 days or longer.[54] These findings suggest that inhaling protective antibody from a portable nebulizer just before an attack might provide some protection in nonimmune individuals. However, the window of opportunity for treatment by intravenous administration or inhalation of specific antibody after exposure is probably minimal at best.

Supportive and Specific Therapy

The route of exposure for any agent is an important consideration in determining prophylaxis and therapy. For oral intoxication with ricin, supportive therapy includes intravenous fluid and electrolyte replacement and monitoring of liver and renal functions. Standard intoxication principles should be followed. Gastric lavage, if not contraindicated, may help to remove the toxin. Activated charcoal should be considered. The degree of adsorption of ricin by activated charcoal is unknown, and it may be minimally effective given the molecular size of the toxin. Percutaneous exposures require judicious use of intravenous fluids and monitoring for symptoms associated with VLS, including hypotension, edema, and pulmonary edema. Supportive care should entail correction of coagulopathies, respiratory support, and monitoring for liver and renal failure. For inhalational intoxication, supportive therapy to counteract acute pulmonary edema and respiratory distress is indicated. Symptomatic care is the only intervention presently available to clinicians for treating incapacitating or potentially lethal doses of inhaled ricin. Positive-pressure ventilator therapy, fluid and electrolyte replacement, antiinflammatory agents, and analgesics would likely be of benefit in treating the aerosol-exposed patient. A variety of chemotherapeutic agents—including cellular membrane effectors,

calcium channel–blocking agents, sodium-calcium exchangers, reducing agents, antioxidants, effectors of endocytosis, nucleoside derivatives, antibacterials, ricin analogs, effectors of cellular metabolism, and binding inhibitors—have been systematically screened in in-vitro and in-vivo models for efficacy against ricin toxicity. However, no compounds were identified that could protect against lethality *in vivo*, and only two compounds, dexamethasone and difluoromethlyornithine, extended survival times in mice.[55,56]

SUMMARY

Ricin is a type II RIP toxin derived from the castor bean plant *R communis*. The plant is globally distributed in tropical and subtropical climates, and the beans are a major agricultural commodity in several countries. This agricultural production, coupled with the ease of toxin extraction, results in the potential availability of large quantities of ricin. Ricin was developed as an aerosol biological weapon by the United States and its allies during World War II, although it was never used in battle. In recent years, the threat of ricin on the battlefield has diminished, while its threat as a potential weapon of bioterror has increased. Although toxic by several routes, the greatest physiological threat is by inhalation. Contamination of the food supply is a lesser threat due to much lower potency by this route.

Signs and symptoms of ricin exposure are route- and dose-dependent. Inhalation probably causes death by hypoxia secondary to massive pulmonary edema and alveolar flooding. Diagnosis is based upon both epidemiological and clinical parameters; laboratory confirmation of clinical samples is possible by immunoassay but complicated by pharmacokinetic factors. Treatment is purely supportive. Prophylaxis will be best accomplished by vaccination, although no vaccine is currently available. However, excellent vaccine candidates based upon genetically-engineered recombinant A chains are currently in advanced development or clinical trials.

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