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Ricin poisoning and forensic toxicology†

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Ricin is one of the most fascinating poisons due to its high toxicity: as little as 500 μg can kill an adult. It gained fame by its use in the so-called 'umbrella murder' to kill the Bulgarian dissident Georgi Markov in 1978. Ricin also became known as a potential bio-terror agent to which people could be exposed through the air, food, or water. The origin, biochemistry, toxicity, and analytical procedures for the determination of ricin are summarized. The homicide of Markov is described as well as recent cases of criminal ricin use. Copyright © 2009 John Wiley & Sons, Ltd.

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Introduction

Ricin is one of the most potent naturally occurring toxins and is among the deadliest poisons available. As little as 500 μg can kill an adult. In forensic toxicology the story about Georgi Markov, who was killed by ricin fired from an altered umbrella, is one of the more well-known cases of homicide worldwide and fascinates due to its association with spying. Ricin has also gained widespread attention as a potential bio-terror agent or weapon of mass destruction. People could be exposed to it through the air, food, or water.

There are a few excellent reviews about ricin toxicity.[1–4] A short overview of work on ricin now follows, focusing mainly on forensic toxicological aspects.

Origin

Ricin derives from the castor bean plant *Ricinus communis* and is easily purified from castor-oil manufacturing waste. Reports on the ricin content of castor beans range between 1% and 5%.[1,5] The process for extracting ricin is well known and has been described in a patent. The patent was removed from the United States Patent and Trademark Office (USPTO) database in 2004 but is still available online.[6] The extraction method is very similar to the preparation of soy protein isolates. No ricin remains in the oil, and ricin is inactivated during oil extraction under heated conditions. It can be inactivated at a temperature of 80 °C in aqueous solution for 1 h, but inactivation requires a higher temperature if it is in powder or crude form.[7] Purified ricin is a white powder that is soluble in water and stable over a wide pH range.

Ingested castor beans are generally toxic only if ricin is released through mastication or maceration.[8] The roots, leaves and seeds of the plants are used in traditional or folk remedies throughout the world.[9]

Structure and Biochemistry

Ricin is a heterodimeric type-2 ribosome-inactivating protein (RIP), whereas a type 1 RIP consists of a single enzymatic protein chain. Type 2 RIPs consist of an A chain, a ribosome-inactivating enzyme (32 kDa) that is functionally equivalent to a type 1 RIP, covalently connected by a disulfide bond to a galactose/N-acetylgalactosamine-binding lectin (34 kDa), also called the B chain. The B chain is catalytically inactive, but serves to mediate entry of the A-B protein complex into the cytosol.

The tertiary structure of ricin has been shown to be a globular, glycosylated heterodimer (Figure 1). The A chain is a N-glycoside hydrolase composed of 267 amino acids with three structural domains with approximately 50% of the polypeptide arranged into eight alpha-helices and eight beta-sheets.[10–12] The three domains form a pronounced cleft, which is the active site marked by the substrate adenine ring. The B chain is a lectin composed of 262 amino acids. It is shaped like a barbell and is able to bind terminal galactose residues on cell surfaces.[13] The ability of ricin to enter the cytosol depends on hydrogen bonding interactions between chain B amino acid residues and complex carbohydrates on the surface of eukaryotic cells containing either terminal N-acetyl galactosamine or beta-1,4-linked galactose residues. The mannose-type glycans of ricin are also able to bind cells that express mannose receptors.[14] It has been demonstrated that 10^6 to 10^8 ricin molecules may bind per cell.[15] However, just a single ricin molecule that enters the cytosol can inactivate over 1.500 ribosomes per minute and kill the cell. The profuse binding of ricin to surface membranes allows internalization with all types of membrane invaginations. Vesicles shuttle ricin to endosomes that are delivered to the Golgi apparatus. Because ricin is stable over a wide pH range, degradation in endosomes or lysosomes offers little or no protection against ricin. Ricin molecules are thought to follow retrograde transport through the Golgi apparatus and enter the endoplasmic reticulum (ER). The A chain inhibits protein synthesis by irreversibly inactivating ribosomes by binding and depurination of a specific adenine from the 28S eukaryotic ribosomal RNA loop contained within the 60S subunit. The adenine ring becomes sandwiched between two tyrosine rings in the catalytic cleft of the enzyme and is hydrolyzed by the

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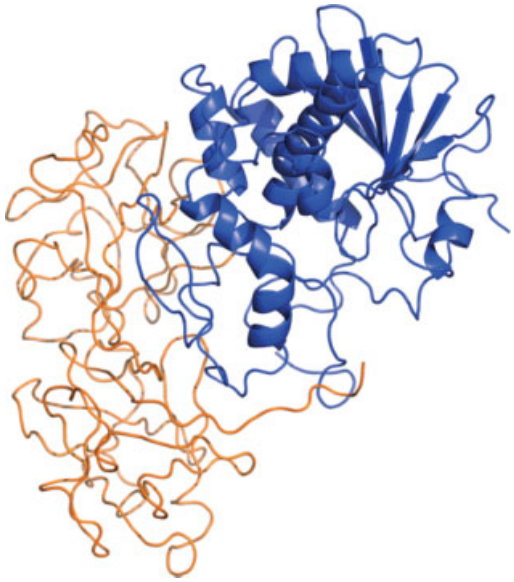

Figure 1. Ricin structure according to Rutenber *et al*.[12] The **A** chain is shown in blue and the **B** chain in orange. Source: http://en.wikipedia.org/wiki/File:Ricin_structure.png (author: Aza Toth).

N-glycosidase action. This process prevents chain elongation of polypeptides and leads to cell death.[16–22] Besides inhibition of protein synthesis, other mechanisms have been noted including apoptosis pathways, direct cell membrane damage, alteration of membrane structure and function, and release of cytokine inflammatory mediators.[3] The mechanism of ricin toxicity is demonstrated in Figure 2.

Many plants, such as barley, have the A chain but not the B chain. People do not become ill from eating large amounts of such products, as ricin A is of extremely low toxicity as long as the B chain is not present. Both type 1 and type 2 RIPs are functionally active against ribosomes *in vitro*. However, only type 2 RIPs display cytoxicity due to the lectin properties of the B chain. In order to display its ribosome-inactivating function, the ricin disulfide bond must be reductively cleaved.[23]

Potential Medical Use

It was suggested that malignant cells are more susceptible to ricin toxicity because they express more carbohydrate-containing surface-lectine binding sites than non-malignant cells. Antibody-conjugated ricin therefore targets cancer cells and has been investigated as an immunotherapeutic agent. In combination with monoclonal antibodies directed at cell surface receptors of tumourous cells, ricin fulfils the role of Erlich's 'magic bullet' in the treatment of certain malignancies.[22,24] Such immunotoxines have recently been improved by means of pegylation.[25] Ricin has also been used in the research into neurological degenerative disorders and in the treatment of intractable painful neuropathies by use of 'suicide transport' in neurons.[26]

Signs and Symptoms of Ricin Exposure

The major symptoms of ricin poisoning depend on the route of exposure and the dose received, although many organs may be affected in severe cases (Table 1).[3,4,8] Initial symptoms of ricin poisoning by inhalation may occur within 8 h of exposure. Following ingestion of ricin, initial symptoms typically occur in less than 6 h. Clinical symptoms typically progress over 4 to 36 h. Patients who remain asymptomatic for 12 h after exposure are unlikely to develop toxicity. However, a possibility of delayed respiratory symptoms at 20 to 24 h after ricin inhalation was experimentally demonstrated in monkeys.[27] Death from ricin poisoning could take place within 36 to 72 h of exposure, depending on the route of exposure (inhalation, ingestion, or injection) and the dose received.

Table 1. Overview of ricin detection methods based on Lubelli *et al*.[51] and Ler *et al*. [86] with no demand for completeness

Detection technique	Limit of detection (ng/ml)	Reference
Quartz crystal microbalance sensors	5000	[52]
Fluoroimmunoassay	1000	[53]
Biosensor assay	320	[54]
Lateral flow devices	250	[55]
ELISA	80	[49]
Inhibition of lysozyme	80	[56]
Immunochromatographic assay	50	[44]
Immunocapture coupled with LC-MALDI MS	50	[57]
ELISA with colorimetric measurement	40	[39]
Microarray biosensor assay	10	[58]
Immunoassay	5	[59]
ELISA	5	[55]
Fluoroimmunoassay	1	[60]
Immunochromatographic test	1	[61]
Biosensor assay	0.5	[46]
Microelectromechanical sensors assay	0.4	[62]
Sandwich avidin/biotin ELISA	0.2	[41]
Mircroarray biosensor assay	0.18	[63]
Biosensor assay	0.1	[43]
Immunoassay on gel-based microchips	0.1	[45]
Chemoluminescence ELISA	0.1	[40]
Protein array	0.1	[64]
Immunocapture coupled with LC-ESI-MS/MS	0.1	[65]
Radioimmunoassay	0.05–0.1	[37]
Bioassay	0.01–0.1	[66]
Luciferase-based assay	0.001	[67]
Piezoelectric detection	10 µg/crystal	[68]
Immuno-polymerase chain reaction	0.00001	[51]

Ingestion

The median lethal dose (LD_{50}) in mice is 30 mg/kg, or approximately 1000-fold higher than by injection or inhalation. Considering reports of human castor bean ingestion, the lethal dose in humans has been estimated to be 1 to 20 mg/kg of body weight. Due to variation in size, weight and content of the beans, an estimation of the ricin dose from the number of beans ingested

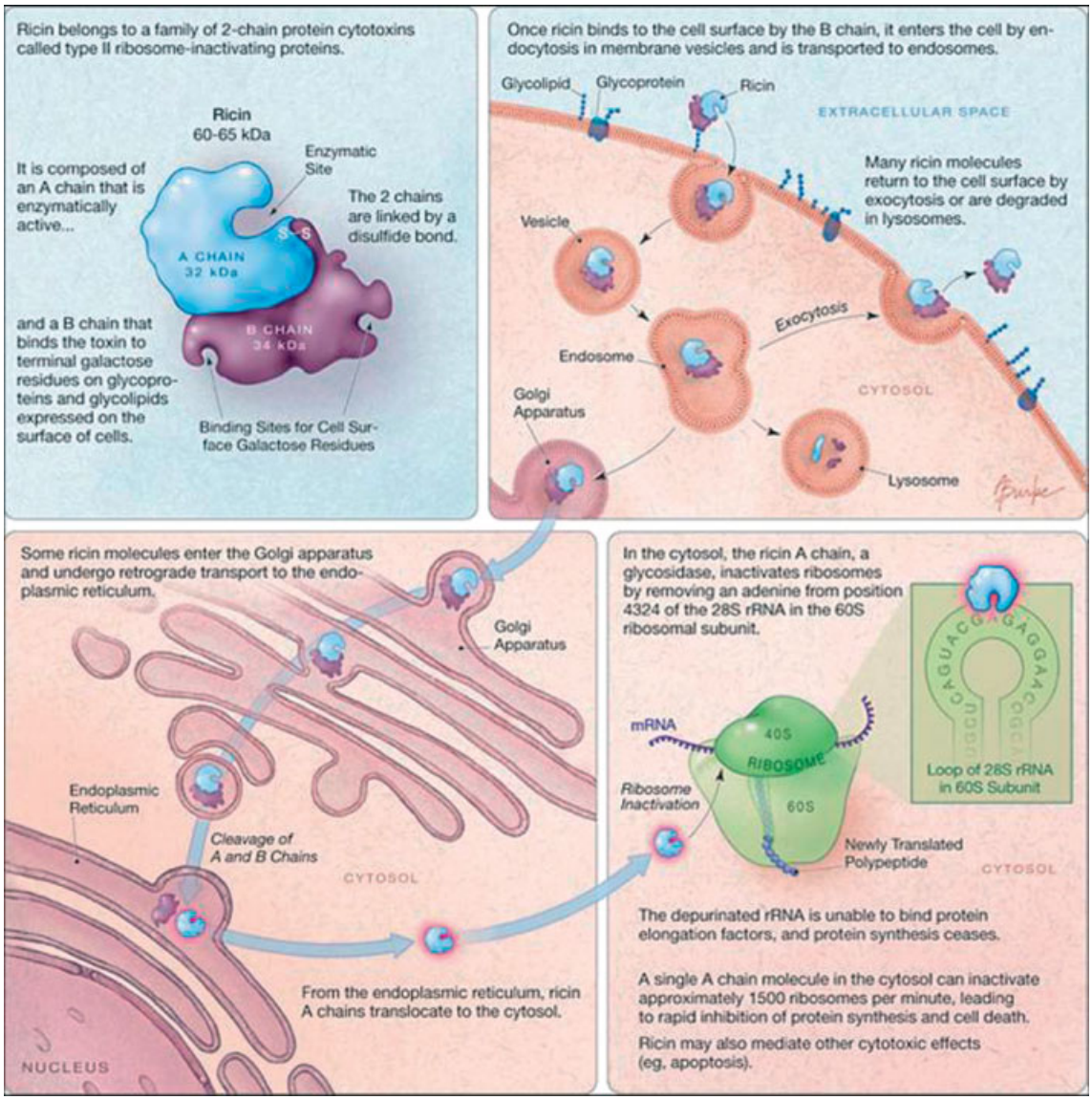

Figure 2. Mechanism of ricin toxicity. Reproduced with permission from: J. Audi *et al*., Ricin poisoning – a comprehensive review, *JAMA* **2005**; *294*, (18), 2345. Copyright © 2005, American Medical Association. All rights reserved.

is inaccurate. Therefore, the number of beans ingested in cases reporting mild to lethal clinical symptoms range from one-half to 30 and the minimum number of beans associated with death was two.[8,28] If a significant amount of ricin was swallowed, vomiting and diarrhoea, which may become bloody, developed.[29] Severe dehydration may be the result, followed by low blood pressure. Other signs or symptoms may include hallucinations, seizures, and blood in the urine. Within several days, the liver, spleen, and kidneys might stop working, possibly resulting in death. There is no report of poisoning from ingestion of purified ricin. Diffuse intestinal hemorrhagic lesions as well as histology consistent with the appearance of apoptotic cell death were found in post-mortem cases.[3]

Inhalation

Within a few hours of inhaling significant amounts of ricin, the likely symptoms would be respiratory distress (difficulty breathing), fever, cough, nausea, and tightness in the chest.[29] Heavy sweating may follow as well as fluid building up in the lungs (pulmonary oedema). This would make breathing even more difficult, and the skin might turn blue. Excess fluid in the lungs would be diagnosed by x-ray or by auscultation with a stethoscope. Finally, low blood pressure and respiratory failure may occur, leading to death. In cases of known exposure to ricin, people having respiratory symptoms that started within 12 h of inhaling ricin should seek medical care. Lung deposition and lethality is significantly influenced by particle size.[30–32] The LD_{50} in mice exposed to particle sizes less than 5 μm is about 3 to 5 μg/kg. Toxicity results from the inhibition of protein synthesis, release of cytokine mediators, and direct injury to the epithelial membrane.

Skin and eye exposure

Ricin is unlikely to be absorbed through normal skin. Contact with ricin powders or products may cause irritation and pain of the skin and the eyes.[29]

Injection

The LD_{50} in mice is approximately 5 to 10 μg/kg and the minimal lethal dose range from 0.7 to 2 μg/kg.[3] Nonspecific clinical symptoms like fever, headache, anorexia, hypotension or abdominal pain can be delayed for as long as 10 to 12 h, even with high doses,[33–35] and may progress to multisystem organ failure.[1,5] Laboratory abnormalities include elevated liver transaminases, amylase, and creatinine kinase, hyperbilirubinemia, myoglobinuria, and renal insufficiency.[3,36] Post-mortem findings include focal haemorrhage in the intestines, brain, myocardium, and pleura. Lymph nodes, kidneys, and intestines may also demonstrate necrosis, hemorrhage and oedema.[3]

Treatment

Treatment for ricin exposures is largely symptomatic and supportive.[3,8] To prevent further systemic absorption, a single-dose of activated charcoal and/or gastric lavage should be considered. Ricin is not susceptible to dialysis and there is no available antidote. A patient with oral ricin poisoning should be given perfusion by aggressive fluid resuscitation, vasopressor therapy, and replenishing of electrolytes. Monitoring and treatment for any evidence of myoglobinuria and renal failure is also indicated. For inhalation exposure treatments include oxygen, bronchodilators, endotracheal intubation, and supplemental positive end-expiratory pressure as needed.

Analytical Aspects

Beside the detection in biological fluids or tissues, the determination of ricin in environmental samples, beverages or food matrices is of interest when it is partially purified or refined into a terrorist or warfare agent.

One of the earliest detection techniques for low concentrations was radioimmunoassay (RIA), which could be used to quantify amounts as low as 100 pg of ricin based on rabbit antiserum buffer.[37,38] Enzyme-linked immunosorbent assay (ELISA) involved shorter assay time and had the advantage that it did not require handling of, and exposure to, radioisotopes. A sandwich ELISA using rabbit anti-ricin antibody was developed with a limit of detection (LOD) of 40 ng/ml for ricin in body fluids through colorimetric measurement.[39] Enhanced colorimetric and chemiluminescence ELISA were explored with affinity-purified goat polyclonal antibodies in a sandwich format giving a LOD of 100 pg/ml for ricin in buffer as well as in human urine and serum.[40]

Early in 1988 a sandwich ELISA was developed that could detect ricin in body tissues to a limit sensitivity of about 200 pg/ml following intramuscular injection.[41] It was recently demonstrated that this assay was sufficiently sensitive to detect measurable quantities of ricin in tissues following administration by pulmonary and oral routes up to 48 h after exposure.[42]

The lengthy assay time – due to several washing steps – and the limited throughput of classical ELISA was the reason for the introduction of laser-induced fluorescence detection, with a LOD of 100 pg/ml in buffer and 1 ng/ml in river water and an assay time of 20 minutes.[43] The use of colloidal gold particles allowed an analysis time of less than 10 minutes with a LOD of 50 ng/ml ricin in a buffer based on monoclonal antibodies and could enhance further to 100 pg/ml with the use of silver enhancer.[44] The advantages of these gold particles were their reduced aggregation, superior mobility and commercial availability. Generally, ELISA shows high sensitivity. However, the major limitation of these assays is the detection of both functional and non-functional ricin.

Alternatives to immunoassays have been developed, such as a hydrogel-based protein microchip[45] or array biosensors, providing the possibility of analysing several toxins in the same sample.[46,47]

Ricin has been detected by electrophoresis using an immuno-gold silver staining (IGSS) procedure to show the presence of the poison in muscle tissue from the injection site of dead victims. Dots were stained using the IGSS method, which was found able to detect less than 10 pg of ricin.[48]

As described above, the identification of ricin in serum, urine, and tissues is typically by ELISA, RIA or by portable biosensors with LODs in the range of 0.05 to 10 ng/ml.[31,37,40,46,49] Recently, a new portable diagnostic device for detecting various biological toxins in body fluids was introduced.[50] The microfluid chip-based immunoassay works rapidly ($<$ 20 min), requires minimal sample volume ($<$ 10 μl) and shows an appreciable sensitivity ($<$ 20 nM and $<$ 10 pM after preconcentration (0.64 ng/ml)) and dynamic range. Such devices would be very useful in the case of the mass exposure of a large population to a biotoxin weapon.

Lubelli *et al*.[51] described the use of the immuno-polymerase chain reaction (IPCR) for the detection of ricin in buffer solution as well as in human serum samples IPCR is a very sensitive antigen detection method combining the specificity of immunological analysis with the exponential amplification of PCR. As a result, the limit of detection (LOD) of an ELISA is generally enhanced 100- to 10 000-fold by the use of PCR as a signal amplification system. The assay allowed the detection of ricin in human serum with a LOD of 10 fg/ml. A list of detection methods used to reveal and detect ricin is reported in Table 2.

The use of capillary electrophoresis (CE) allows both rapid separation and rapid purification of complex mixtures. Coupled with mass spectrometry (MS) it allows characterization of and differentiation between various forms of ricin toxin.[69,70]

Darby *et al*.[71] described liquid chromatography-electrospray/mass spectrometry (LC-ES/MS) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS methods for the identification of ricin toxin and an additional alkaloid marker, ricinine, from crude plant material. They developed a general protein identification scheme for size classification followed by the analysis of the tryptic digest. Fragments of the digest can be searched in an online database for tentative identification of unknown proteins followed by comparison to authentic reference material. The identification of the alkaloid ricinine by GC/MS and LC/MS was shown to be a complementary technique for the determination of castor bean extracts.

Fredriksson *et al*.[72] demonstrated that ricin can be identified by LC-ES-MS/MS experiments with reduced, cysteine-derivatized, trypsin-digest material. It was also shown that MALDI MS can be used to prove the presence of intact ricin and to screen samples for ricin peptides. To improve the method's sensitivity and efficiency, a few marker peptides from the A and B chain were selected to establish an LC-MS/MS screening method. The specific trypsin digest peptides T5, T7, T11, T12 and T13 from the A chain and T3, T5, T14, T19, and T20 from the B chain were chosen and this procedure allowed a single analysis run per sample at maximum instrument detection sensitivity. As described by Östin *et al*.,[73] the use of organic solvent to assist trypsin digestion dramatically shortens the sample preparation time from an overnight digestion

to a 1 h digestion. The response time for an unambiguous answer regarding the presence or absence of ricin in a sample is shortened from 24 h to 2 h. Furthermore, this procedure leaves the disulfide bonds in the protein intact, which indicates the presence of an intact toxin and provides additional forensic evidence for the analytical results.

Becher *et al*.[65] described an approach based on immunocapture by anti B-chain antibodies coupled to MS determination of the release of adenine by the A chain, which also allowed the sensitive and specific determination of the entire active toxin with a LOD of 0.1 ng/ml. This was the first method specifically detecting functional ricin with a sensitivity similar to that of immunoassays and easily applicable to environmental samples. The assay required 26 h, but a preliminary response can be given after 6 h with threefold lower sensitivity. Nevertheless, a false-positive result is still possible through the nonspecific extraction of other RIPs, especially when working on complex environmental samples.

Duriez *et al*.[57] also used immunocapture based on this procedure to isolate ricin from liquid-based samples. Magnetic beads coated with protein G were used to attach monoclonal antibodies, which were specifically directed against the B-chain of ricin. When the sample was passed over the beads, only ricin was attracted to the antibodies. Other components of the sample remained in the solution. Ricin was eluted with a weak solution of trifluoroacetic acid. The extract was neutralized, then ricin was digested with trypsin to produce a mixture of peptides. The digestion was not conducted under typical proteomics conditions but in a mixture of acetonitrile and water, the added organic solvent giving higher specificity of trypsin and a greater digestion yield. In contrast to the previous procedure, MALDI MS was used for the detection and mass spectra of the resulting peptide mixture revealed 20 peptides. From these a panel of three was selected for detection, corresponding to the 161–169, 150–160 and 233–248 residues of the protein. High selectivity and good detection sensitivity were demonstrated, without interference from other peptides in the mass spectrum. The sequences were subjected to similarity searches to ensure that they were specific to ricin. In order to ensure accurate detection and to allow for peptide quantification, synthetic stable isotope-labelled analogues of the three peptides were added to the eluted ricin before tryptic digestion. Under these conditions, a detection limit of 50 ng/ml was obtained in a buffer solution spiked with ricin. This is higher than for existing immunoassay or bioassay methods, but is sufficient to measure the levels of ricin in environmental and food samples. For ricin detection in milk, the presence of lactose might present a problem because it is known to bind strongly to the ricin B-chain. However, for semi-skimmed and skimmed milk spiked with ricin, the mass spectrometric intensities of the three peptides were 85–110 and 110–125% of those observed in buffer, showing that lactose did not inhibit ricin binding to the antibody. The method was tested on the detection of ricin in castor beans from different varieties of the genus *Ricinus communis* or from different geographical origins (Spain, Tanzania, Pakistan, India, China). In all cases, the antibody recognized the B-chain and the three diagnostic peptides were observed consistently. This implies that there are no apparent differences between the peptide sequences of the ricins from the different origins or cultivars. The researchers claim that this is the first proteomics-based method for the analysis of ricin in environmental samples. The overall analysis time is about 5 h, which is regarded as adequate for bio-terrorism incidents.

As described above, the use of the alkaloid ricinine as a biomarker in crude plant material was proposed a few years ago and the small size of the molecule (164 g/mol) allows a simple extraction procedure followed by GC/MS or LC/MS analysis. According to

Table 2. Clinical symptoms and progression of disease associated with ricin poisoning by route of exposure. Source: Department of Health and Human Services[4]

Route of exposure	Common clinical symptoms	Progression of disease
Inhalation	• Rapid onset of irritation of nose and throat • Respiratory distress possibly leading to respiratory failure • Dyspnoea (difficulty breathing) • Pulmonary oedema • Flu-like symptoms of fever, weakness, nausea, myalgia (muscle pain), or arthralgias (aches and pains in joints)	• Cough, difficulty breathing, flu-like symptoms within 4–8 h • Hypotension and pulmonary edema within 18–24 h • Death may occur within 36–72 h
Ingestion	*Mild:* • Nausea, vomiting, diarrhoea, abdominal cramping and pain *Moderate to severe:* • Persistent vomiting and voluminous diarrhoea (bloody or non-bloody) • Dehydration and hypovolemic shock • Hepatic and renal failure possible • Mild haemolysis (not requiring blood transfusions) • Liver and kidney dysfunction	• Rapid onset of nausea, fever, and abdominal cramps within 1–6 h • GI symptoms may occur as late as 10 h • In mild cases, the symptoms often resolve in 24 h • In severe cases death may occur within 36–72 h
Parenteral (injection)	• Flu-like symptoms with fatigue and myalgias • Local necrosis of muscles and regional lymph nodes at injection site • Pain at injection site • Weakness, fever and/or vomiting • Shock • Multisystem organ failure	• Weakness or pain at site of injection within 5 h • Fever and vomiting within 24 h • Death may occur within 36–48 h

Johnson *et al*.[74] ricinine can be detected easily in urine after solid-phase extraction and LC-ES isotope dilution MS-MS with a limit of quantification of 0.083 ng/ml. The method was applied to an animal study, to a crude ricin preparation scheme, and to a forensic analysis of human urine. Ricinine was measured in rat urine at least 48 h after exposure and could be found in human urine after a lethal exposure to ricin. Assumed 48 h after injection of a ricin preparation with intend to suicide, ricinine was found in a concentration of 4.24 ng/ml. It has been proposed that, 48 h after a lethal exposure to a ricin preparation, ricinine is expected at concentrations ranging from 0.08 to 10 ng/ml. According to the authors any level below 0.08 ng/ml should imply a less-than-lethal exposure. The same method is also useful for the detection of abrin. Measurements of the background urine level of ricinine in the general population resulted in 2% positive cases ($<$ 4 ng/ml), indicating an (actual) exposure to a product made from castor oil products.

However, ricinine concentrations should be interpreted cautiously. In a 75 kg male who consumed 6 castor seeds prior to seeking medical treatment with non-life-threatening symptoms, urinary ricinine concentrations were quantified for up to 63 h post-exposure with decreasing concentrations until 130 ng/ml.[75] Thus analytical data should only be used to identify those who are exposed and those who are not exposed. Recently, there was a case of a non-lethal multisystem organ failure after large-volume subcutaneous injection of castor oil for cosmetic enhancement, when an unlicensed practitioner injected 500 ml of castor oil bilaterally to the hips and buttocks of a 28-year-old male-to-female transsexual.[76] Castor oil absorption was inferred from recovery of the biomarker ricinine in the patient's urine in a concentration of 41 ng/ml 12 h postinjection. Fatal ricin toxicosis in a puppy was confirmed by a qualitative LC-MS/MS determination of ricinine in stomach content.[77]

Ricin and the 'Umbrella Murder'

A Bulgarian dissident, Georgi Markov, was killed by a poison dart filled with ricin and presumably fired from an umbrella in London in 1978.[78–80] At about 1.30 p.m. on 7 September as he waited at a bus stop he felt a jab in the back of his right thigh and saw a man picking up an umbrella. When he arrived at his office Markov was in great pain and showed a colleague an 'angry red spot, like a pimple' on the back of his thigh. There was also blood on his jeans. At home within a few hours Markov became very sick, developed a high temperature and vomited. The next day at 11.13 p.m. he was admitted to a hospital. There it was noted that he looked hot and very ill, his pulse was fast but regular and his blood pressure was normal. Swollen lymph glands were noted in his right groin and an examination of his right thigh showed a circular 6 cm diameter area of inflammation with a central puncture mark of 2 mm diameter. The patient's condition declined rapidly with a fall in blood pressure and a tentative diagnosis of septicaemia was made. Next day his pulse rose to 160/min and his temperature fell. He was cold, sweating and dizzy and his white cell count had risen considerably. He stopped passing urine and vomiting became pronounced featured, with blood in the vomit. On 11 September an electrocardiogram showed complete block of the conduction system. Cardiac arrest occurred at 9.45 a.m. and death was confirmed at 10.40 a.m. on the fourth day after the injury.

During post-mortem examination a single metallic sphere, 1.53 mm in diameter, was found subcutaneously in the thigh, composed of an alloy of 90% platinum and 10% iridium. It had two tiny holes with a diameter of 0.34 mm each. The volume available for the retention of a toxic agent was thus only of the order of 0.28 mm^3. No specific isolation of any poison was possible. It was assumed that a sugary substance coated the tiny holes, creating a bubble that trapped a poison inside the cavities. This coating was designed to melt at 37 °C (body temperature). As the pellet was shot into Markov, the coating melted and the poison was free to be absorbed into the bloodstream and kill him. Based on the symptoms and the extraordinary high toxicity for such a small dose, ricin was the only choice. An experiment with a pig has been described,[79] which had been injected with a somewhat greater dose than Markov had received. With an illness similar to Markov's, the animal died after 26 h. Thereafter the coroner was satisfied that Markov had been unlawfully killed by a tiny pellet containing a 0.2–0.5 mg dose of the poison ricin. In Figure 3 a diagram of a possible umbrella gun is demonstrated. The umbrella itself was not recovered and nobody really knows how it worked: it is purely a matter of hypothesis.

Markov's assassination was detected only because the pellet carrying the poison had not dissolved as expected. His assassin has never been captured despite close cooperation between British and Bulgarian authorities, including Interpol.[81] It was believed that the operation was supported by the technical staff of the Soviet KGB and seemed to have involved many senior members of the Bulgarian secret police. In June 1992 General V. Todorov, the former intelligence chief, was sentenced to 16 months in jail for destroying 10 volumes of material on the case. A second suspect, General Savov, committed suicide rather than face trial for destroying the files. Another Bulgarian spy, Kotsev, who was

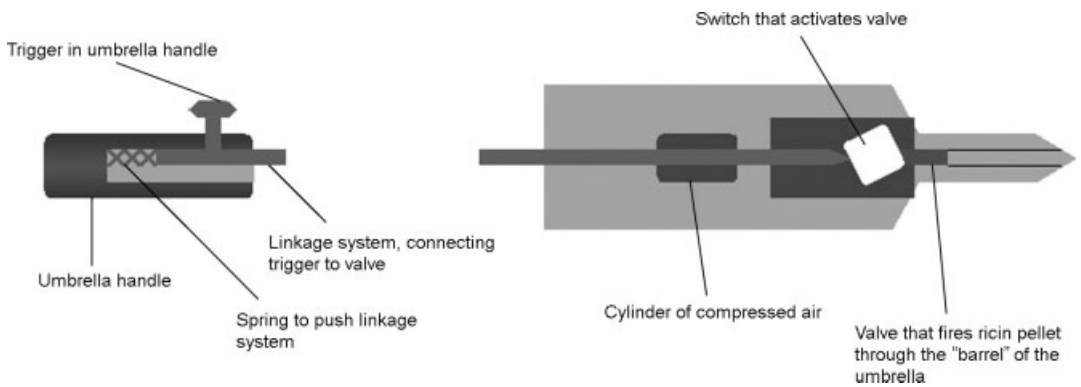

Figure 3. Diagram of the firing mechanism of the umbrella used to assassinate Georgi Markov according to Fleming[80].

widely believed to have been the operational commander of the Markov assassination plot, died in an unexplained car accident. According to Scotland Yard the case remains open.

Further Cases of Criminal Ricin Use

The US Chemical Warfare Service began studying the poison as a potential weapon of war as early as 1918 during the First World War. During the Second World War a ricin bomb was developed by the British military. Ricin was then code-named Compound W. The weapon, dubbed the W-bomb, was tested but never used on soldiers or civilians. Recently, the toxin has found its way into the arsenals of extremist individuals, groups and governments.[82–84]

In the US, four members of the Patriots Council, an extremist and anti-government group, were arrested in 1991 for allegedly plotting to kill a US marshal with ricin. They planned to mix the agent with a solvent and then smear it on the door handles of the victim's vehicles.

There have been a few more cases recently:

June 2002, US: A man was arrested for possession of 1 g ricin.

August 2002, Iraq: A primitive testing facility, run by members of a Kurdish Sunni Islamist group, was discovered. Authorities said that ricin had been tested on barnyard animals there.

January 2003, UK: Scotland Yard arrested seven terror suspects after traces of ricin were discovered at their homes. Four of the men were charged with terrorism offences under Britain's Terrorism Act 2000 and with 'being concerned in the development or production of chemical weapons' under the Chemical Weapons Act of 1996.

March 2003, France: Small bottles containing traces of ricin were found in a Paris train station, according to French police.

October 2003, US: Ricin was found in a sealed envelope in a postal handling facility in Greenville, South Carolina.[85]

November 2003, US: The Secret Service intercepted a letter, addressed to the White House, which contained a vial of ricin. The letter, signed by 'Fallen Angel', complained about trucking regulations and was nearly identical to one discovered in South Carolina.

February 2004, US: Ricin was found in the mailroom of the Dirksen Senate Office building in Washington DC. The mailroom handled correspondence addressed to the Senate Majority Leader Bill Frist and others. No one became ill.

January 2005, US: In Ocala, Florida, a man with no known ties to terrorists or extremists is arrested by the FBI after agents found ricin in the home he shared with his mother.

October 2006, US: In Phoenix, Arizona, a man was sentenced to 7 years in prison for the attempted manufacture of ricin.

May 2007, Ireland: Traces of ricin had been found in an Irish prison cell. It was smuggled into Ireland from the US in a contact lens case, to be used in an assassination plot.

February 2008, US: A man who stayed in a Las Vegas hotel room where ricin was found was suffering from respiratory distress and was transported to a hospital.

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