MMSL
Military Medical Science Letters
Since 1925

Mil. Med. Sci. Lett. (Voj. Zdrav. Listy) 2018, 87(4), 158-168
ISSN 0372-7025 (Print)
ISSN 2571-113X (Online)
DOI: 10.31482/mmsl.2018.027

REVIEW ARTICLE

HIGHLY TOXIC RIBOSOME-INACTIVATING PROTEINS AS CHEMICAL WARFARE OR TERRORIST AGENTS

Jiri Patocka [1,2]

[1] Institute of Radiology, Toxicology and Civil Protection, Faculty of Health and Social Studies, University of South Bohemia České Budějovice, České Budějovice, Czech Republic
[2] Biomedical Research Centre, University Hospital, Hradec Kralove, Czech Republic

Received 20th March 2018.
Accepted 24th April 2018.
Published 7th December 2018.

Summary

Biological weapons include infectious agents and toxins. Toxins are poisons produced by living organisms. An important group of toxins are ribosome inactivating proteins (RIPs) of plant or microbial origin that inhibit protein synthesis by inactivating ribosomes. RIPs have been of great scientific interest due to their importance in human health, as both pathogenic agents and therapeutics, but also due to their potential use in biological warfare and bioterrorism. RIPs relevant to bioterrorism include mainly ricin and abrin. Ricin is protein produced in the seeds of the castor oil plant (*Ricinus communis*). Abrin is protein that has been isolated from the seeds of *Abrus precatorius*. Both inactivate ribosomes, which results in toxicity because of the inhibition of protein synthesis. Abrin and ricin are substances very toxic to humans in all types of administration, with the exception of oral administration. Symptoms include nausea, diarrhea, tachycardia, hypotension, and seizures. Treatment is supportive, and no antidote exists.

Key words: Plant toxic proteins; ribosome inactivating proteins (RIPs); abrin; ricin; modeccin; viscumin; volkensin; warfare; medicine

INTRODUCTION

Many plants produce proteins that are today referred to as ribosome-inactivating proteins (RIPs) (Stirpe and Battelli, 2006). Some of these RIP-expressing plants are very toxic and their toxicity has been known since antiquity (Olsnes, 2004). Most commonly RIPs are single–chain proteins (type 1 RIPs), but some (type 2 RIPs) possess a galactose–specific lectin domain that binds to cell surfaces. The latter RIPs are potent toxins, the best known of which are abrin and ricin (Patocka, 2001). Because of their high toxicity, viscumin, modeccin and volkensin are also associated with these two proteins. Abrin, ricin, viscumin, modeccin, and volkensin are very potent toxins derived from plants (Patocka and Streda,). They are glycoproteins composed of two polypeptide chains linked by a disulphide bridge. The A-chain is the enzymatic toxic moiety and B-chain is responsible for bonding to the target cell and internalization of toxin.

The A-chain is the enzymatic toxic moiety and B-chain is responsible for bonding to the target cell and internalization of toxin. The B-chain is a lectin-like peptide that has strong affinity for sugar moieties displayed

✉ University of South Bohemia České Budějovice, Faculty of Health and Social Studies, Institute of Radiology, Toxicology and Civil Protection, Jírovcova 24/1347, 370 04 České Budějovice, Czech Republic
toxicology@toxicology.cz

on the surface of cells and helps promote translocation through the plasma membrane. The toxic part of the toxin molecule removes an adenine from a specific adenosine residue in ribosomal RNA and blocks proteosynthesis. That is the reason of extreme toxicity of these compounds and their capacity to be used as biological warfare agents or terrorist weapon (Anderson, 2012). Therefore all these compounds are in the schedules of controlled biological agents and toxins. Contrariwise, plant ribosome-inactivating proteins are studied intensive as possible chemotherapeutic agents (Das et al., 2012). RIPs have antibacterial and antiviral activities, and, in a widespread application, can also be linked to antibodies or ligands to form immunotoxins or conjugates specifically toxic to a given type of cell (Rust et al., 2017).

MOLECULAR STRUCTURE OF TYPE 2 RIBOSOME-INACTIVTING PROTEINS (2 RIPs)

The glycoproteins like abrin, ricin, viscumin, volkensin and modeccin come under the group of toxic lectins of A- and B-chains. The A-chain is an enzyme whereas B-chain is a lectin. Most of the research and information on plant toxic proteins has been obtained from studies on ricin. Ricin is considered the first lectin to be discovered, and it is thus the prototypical lectin in this category (Cummimgs et al., 2017). Chemical structure and the mechanism of toxic action all this ricin-related protein family are very similar (Olsnes et al. 1974). The resemblance of A- and B-chains of all RIPs is evident from the fact that they are interchangeable and hybrid toxin built-up from modeccin A-chain and ricin B-chain was prepared (Sundan et al. 1983).

TOXIC ACTION OF TYPE 2 RIPs

The glycoproteins ricin, abrin, viscumin, volkensin and modeccin are lectins composed of 2 chains, linked by a disulfide bond (Kozlov et al., 2006). A chain inhibits protein synthesis by irreversibly inactivating eukyryotic ribosomes through removal of a single adenin residue from the 28s ribosomal RNA loop and prevents chain elongation of polypeptides and leads to cell death. B chain binds to galactose-containing glycoproteins and glycolipids expressed on the surface of cells and facilitates the entry of toxin into cytosol (Shi et al., 2016). Part of the toxin bound to the cell surface undergoes receptor-mediated endocytosis (Manske et al. 1989) and by this way a RIP is internalized. As soon as the toxin molecule is internalized to vacuolar and tubo-vesicular portions of the endosomal system where most of it remains bound to the plasma membrane, protein is transported retrograde through the Golgi to the endoplasmic reticulum and the A-chain to bind to its target ribosome site and cause toxic effects in the cell. When the ricin A-chain is separated from the B-chain and administered parenterally to amnimals, its toxicity is diminished by > 1,000-fold compared with ricin holotoxin (Soler et al., 1992). The mechanism of toxic action of ricin is schematically illustrated in Figure 1.

Ribosomes are complex structures, composed of protein and nucleic acid (rRNA) components. They are responsible for protein synthesis from mRNA and amino-acid subunits linked to tRNA. Ribosomes have two subunits, a large subunit, which contains an rRNA fragment known as the 60s fragment and a smaller subunit. The 60S fragment is made up of several pieces of RNA, one of which is the 28S rRNA. It is thought to be the RNA components that are most important in protein chain elongation catalysis (Larsson et al. 2002). The A-chain is an N-glycosidase, which removes bases from nucleic acids. It catalytically and irreversibly inactivates the 60S, large ribosomal subunit (Manske et al. 1989) so that modifies a base in the 28S rRNA fragment of the 60S RNA chain and thus halts protein synthesis.

CLINICAL COURSE OF POISONING

Illness symptoms of all RIPs and the course of poisoning are very similar because the mechanism of toxic action of these toxic proteins is identical. Best known is poisoning of human by ricin (Lopez Nunez et al. 2017). People who have poisoned ricin are large numbers (Audi et al. 2005; From et al. 2015). Toxic effects of ricin have a latent period and take 2-24 hours to develop. After ingestion the primary symptoms are abdominal pain, vomiting and diarrhoea often with blood. The toxin causes haemorrhages in the intestine, mesenterium and omentum. It may also cause a diffuse nephritis, multiple necroses in the liver and kidneys with cytoplasmic vacuolation and pyknosis of the nuclei (Knight, 1979). In the myocardium the myofibrils undergo degeneration. Within several days there is severe dehydration, a decrease in urine, thirst, burning throat, headache and the patient may die from hypovolemic shock. The patients' temperature decreases before death, and they often undergo a characteristic shivering. Death occurs

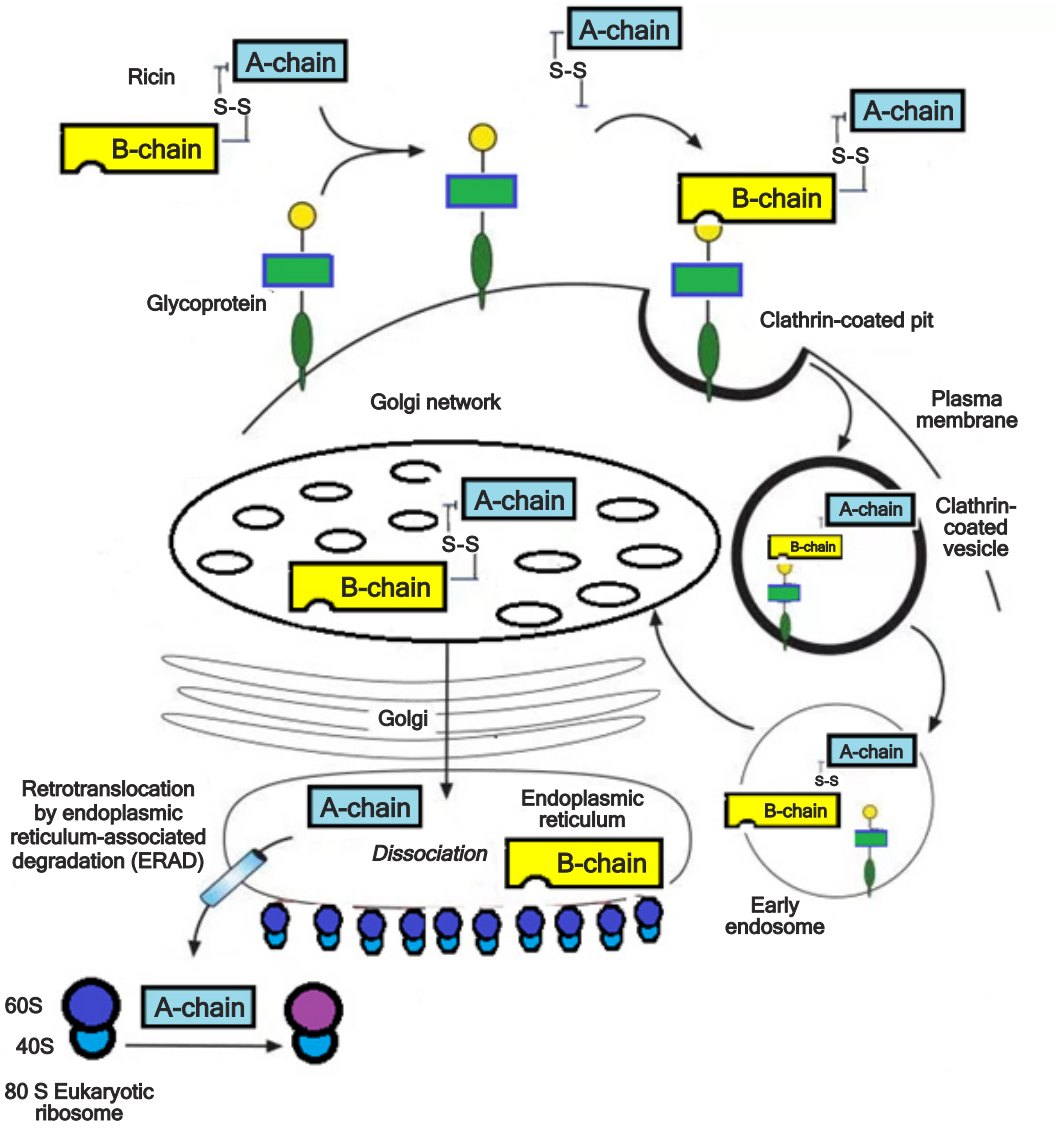

Figure 1. Mechanism of action of ricin.

Ricin binds to glycoproteins of the plasma membrane and internalizes into the cell. A small number of ricin molecules are transported first to the Golgi netwok and then to the endoplasmatic reticulum. The disulphide bridge is reduced and the A-chain translocates to the cytosol by the endoplasmatic reticulum-associated degradation (ERAD) pathway. In the cytosol, the A-chain cleaves an adenine residue (A4324) near the 3'- end of 28S RNA in the 60S subunit. This inactivates ribosome function and blocks protein synthesis and cause cell death.

in exhaustion or cramp (Bradberry et al. 2003). When administered paraenterally ricin is twice as toxic as cobra venom, and is probably the most toxic paraenteral substance in the plant kingdom. After paraenteral administration the patient may present with fever, leucocytosis, and then falling blood pressure and temperature. The primary target organs are the kidney, liver, and pancreas. Differences in toxicities of particular RIPs are not very distinct. From the literary data the most toxic is abrin (Patocka 2001).

DIAGNOSIS

Like other potential intoxications on the unconventional battlefield, epidemiological findings will likely play a central role in diagnosis. The observation of multiple cases of very severe pulmonary distress in a population of previously healthy young soldiers, linked with a history of their having been at the same place and time during

climatic conditions suitable for biological warfare attack, would be suggestive of ricin intoxication. The differential diagnoses of aerosol exposure to ricin would include staphylococcal enterotoxin B, exposure to pyrolysis by-products of organofluorine polymers (Patocka and Bajgar, 1998) or other organohalides, oxides of nitrogen, and phosgene. Diagnosis of an aerosolized attack ricin is similar to that of an attack with staphylococcal enterotoxin B, exposure to pyrolysis by-products of organofluorine polymers (Patocka and Bajgar, 1998) or other organohalides, oxides of nitrogen, and phosgene. Laboratory findings are nonspecific but similar to other pulmonary irritants that cause pulmonary edema. Enzyme-linked immunosorbent assays in blood or other body fluids (Poli et al., 1994) or by radioimmunoassays (Godal et al., 1981) or immunohistochemical techniques may be useful in confirming abrin and/or ricin intoxication but identification in body fluids or tissues is difficult (Garber et al., 2010; Xu et al., 2015).

Recently a first of its kind portable, colorimetric detection system has been developed for the rapid diagnosis of abrin poisoning (Cho and Jaworski, 2014). This unique diagnostic test for abrin poisoning has demonstrated key benefits of portability and simple visual readout. These significant advantages can thus provide the potential for more rapid assessment and corresponding poison management if dedicated toxicology laboratories are not an option. For the convenience of fast measurement in the outdoor environment, a portable electrochemiluminescence biosensor with the screen-printed electrode as the reaction center was developed, which possesses the characteristics of high sensitivity, small scale, simplified operation and so on, and has been used for in situ detection of abrin (Liu et al., 2018).

PROTECTION

The usually chemical protective masks are effective against inhaled ricin as well as against all other RIPs. Two types of tight-fitting masks may be used: 1) respirator with HEPA filters, or 2) respirator with charcoal filters (Audi et al. 2005). A protective mask is the best protection against inhalation. Future protection efforts are aimed at developing a vaccine against inhaled ricin and abrin. No approved vaccine or specific antidote is currently available for human use, but experimental, recombinant vaccines are under development (Pittman et al. 2015; Wang et al. 2015). If exposure to skin occurs, decontamination with soap and water is available. Also, hypochlorite solutions can be used to inactivate ricin (Kent, 2006). As a toxin, ricin and other RIPs acts directly on the individual exposed to it and is not reproduced within the individuals: it cannot be passed from person to person. Quarantine of affected individuals would be of no value.

PROPHYLAXIS

Because RIPs are potential agents for bioterrorism an effective vaccine against RIPs poisoning is urgently required (Griffiths et al., 1995). At present, the potential for vaccine development against RIPs, particularly against abrin and ricin, has been intensively studied (Zhang t al., 2014). Recently, a recombinant vaccine consisting of the A subunits of abrin and its homolog *Abrus precatorius* agglutinin (APA) was demonstrated to protect mice from abrin lethality (Kumar and Karande, 2016). Abrin and ricin are highly potent toxins, and is classified as one of the most important biological warfare and bioterrorism agents. The development of an effective vaccine is impor-tant in the prevention of intoxication by abrin and ricin. There is currently no approved vaccine for therse RIPs.

DECONTAMINATION

Ricin may be inactivated with 0.5% hypochlorite (Kent, 2006). Since it is not dermal active and is involatile, decontamination may not be as critical as with certain other biological and chemical agents (Spivak and Hendrickson, 2005).

RIPs AS CHEMICAL WARFARE OR TERRORIST AGENTS

Toxins have properties of biological and chemical weapons and the same is true for RIPs, especially for ricin and abrin (Kuca and Pohanka. 2010; Anderson, 2012). Both these natural toxins cause multiorgan toxicity by blocking protein synthesis. Ricin is the only toxin to exist naturally in large quantities. It is a byproduct of castor

oil production and ricin isolation is a simple and cheap separation. Easy preparation and low price might make this toxin attractive to poor country. Center of Disease Control and Prevention (CDC) recognized ricin as a biological weapon category B. The lethal dose of ricin toxin after parenteral administration is 0.1 μg/kg and after oral administration 0.2 mg/kg. The first symptoms of poisoning occur within a few hours after application of toxin as a nausea, vomiting and abdominal pain. In the final stage there are observed: cardiac arrhythmia, collapse and symptoms suggestive of involvement of the central nervous system (Patocka and Streda, 2006). Stage immediately preceding death is a state of coma (From and Płusa, 2015). The ricin toxin is still the substance against which action has no optimal antidote.

At the end of the First World War, US initiated a research program with ricin named compound W as a potential replacement of phosgene and during the Second World War, US produced, together with Canada, United Kingdom and France, 1,700 kg of ricin. United Kingdom designed a 500 pounds' bomb with ricin but never used it (Diac et al., 2017). The former Soviet Union was the first to really use ricin as a bioweapon in the cases described before. Even though they tried to develop a bomb, the costs of protecting the toxin from thermal effect was too high incorporate in artillery projectiles but the results were disastrous and the project abandoned. In the last century, at least 30 incidents related to the criminal use of ricin were registered, but in only half of these the toxin was actually identified . Al Qaeda attempted seed extraction of ricin by following methods described in US paramilitary publications but the ricin content of the extract was less than 1%, unsuitable for mass poisoning (Pearson, 1999).

Ricin's significance as a potential biological warfare toxin relates in part to its wide availability. Worldwide, one million tons of castor beans are processed annually in the production of castor oil and in the waste is five percent ricin by weight. The toxin is also quite stable and extremely toxic by several routes of exposure, including the respiratory route. Ricin is said to have been used in the assassination of Bulgarian exile Georgi Markov in London in 1978. Markov was attacked with a specially engineered weapon disguised as an umbrella which implanted a ricin-containing pellet into his body (Crompton and Gall, 1980; Papaloucas et al., 2008). The most likely scenarios in which ricin intoxication might be seen by military medical personnel are small-scale battlefield or terrorist delivery of an aerosol and parenteral administration of the toxin to an individual as an assassin's tool (Madsen, 2001).

Abrin may be considered to be an available toxin for weaponizing because its source, *Abrus precatorius*, may be easily cultivated and the preparation of the pure toxin is not complicated. For nations or terrorists who lack the money to spend on nuclear weapons and other high-tech killing instruments, toxin warfare offers horrific appeal: biological/toxin weapons are cheap, easy to make, and simple to conceal. Even small amounts, if effectively used, could cause massive injuries and make many suffer (Patocka et al. 2007).

ABRIN

Abrin is a potent toxin that has been isolated from the seeds of *Abrus precatorius* (or Rosary pea). Its use as a tool for research was described in 1972 by Sharon and Lis (1972). Abrin exists in two forms, abrin a and abrin b. Both are composed of two chains, an A-chain and a B-chain. A disulfide bond between Cys247 of the A-chain and Cys8 of the B-chain links the A and B chains. The A-chain is 251 residues and is divided into 3 folding domains. The A-chain catalytically inactivates 60S ribosomal subunits by removing adenine from positions 4 and 324 of 28S rRNA therefore inhibiting protein synthesis. The B-chain is a galactose-specific lectin that facilitates the binding of abrin to cell membranes (Olsnes and Pihl, 1976; Chen et al., 1992). The B-chain of both forms of abrin consist of 268 amino acid residues and share 256 identical residues (Kimura et al., 1993). Comparison of their sequences with that of the ricin's B-chain shows that 60% of the residues of abrin's B-chain are identical to those of the ricin's B-chain and that two saccharide-binding sites in ricin B-chain identified by a crystallographic study are highly conserved in abrin B-chain (Kimura et al., 1993).

The mechanism of toxic action of abrin is identical to that of ricin, but the toxicity of abrin in mice is 75 times that of ricin (0.04 μg/kg for abrin compared to 3 μg/kg for ricin.) The diagnosis, clinical features, treatment, protection, prophylaxis and so on is also the same for both abrin and ricin intoxications (Olsnes et al., 1978). Published toxicity data for abrin are summarized in Table I.

Table I. Published toxicity data for Abrin

Organism	Test Type [a]	Route	Report Dose	Reference
mouse	LD_{50}	intraperitoneal	20 µg/kg	Lin et al., 1971
mouse	LD_{50}	intraperitoneal	0,91 µg/kg	Chaturvedi et al., 2015 [b]
mouse	LD_{50}	intravenous	20 µg/kg	Berdy, 1982
mouse	LD_{50}	unreported	20 µg/kg	Tung et al., 1975
rat	LDLo	oral	300 mg/kg	Anonymous, 1955
rabbit	LDLo	oral	21 mg/kg	Anonymous, 1955
human	LDLo	oral	7 µg/kg	Merck Index, 1989

[a] LD_{50} = Acute Lethal Dose, LDLo = Lethal Dose Low

[b] The intraperitoneal LD_{50} value of purified abrin published by Chaturvedi et al (2015) for mice was found to be 0.91µg/kg of body weight. It is not clear why, but this value is much lower than the values published by Lin et al. (1971) and Berdy (1980), respectively. Indian authors (Chaturvedi et al. 2015) could have, for example, a much more purified abrin.

RICIN

Ricin was found by Stillmark in 1889 as the first plant lectin from the seeds of the castor plant, *Ricinus communis*. As with abrin, ricin is a lectin consisting of two polypeptide chains, the A-chain (30 kDa) and the B-chain (32 kDa), linked by a disulfide bond. It is one of a group of dichain ribosome-inactivating proteins, which are specific for the depurination of a single adenosine in ribosomal ribonucleic acid (Barbieri et al., 1993). The A-chain of ricin has the ability to modify catalytically the 28S subunit of ribosomes to block protein synthesis. The lectin subunit, B chain, of ricin plays an important role of binding to the cell surface glycoconjugates of target cells and facilitates the internalization and translocation of the toxin to cytosol (Lord et al., 1994).

The toxicity of castor beans has been known since ancient times, and more than 750 cases of intoxication in humans have been described (Rauber and Heard, 1985). There is a 100-fold variation in the lethal toxicity of ricin for various domestic and laboratory animals, per kilogram of body weight. Of animals tested, the chicken and frog are least sensitive, while the horse is the most sensitive (Balint, 1974). Toxicity of ricin also varies with route of challenge. In laboratory mice, the approximate dose that is lethal to 50% of the exposed population (LD_{50}) and time to death are, respectively, 3 to 5 µg/kg and 60 hours by inhalation, 5 µg/kg and 90 hours by intravenous injection, 22 µg/kg and 100 hours by intraperitoneal injection, 24 µg/kg and 100 hours by subcutaneous injection, and 20 mg/kg and 85 hours by peroral administration. Low oral toxicity reflects poor absorption of the toxin from the gastrointestinal tract. Published toxicity data for ricin are summarized in Table II.

VISCUMIN

Viscumin (Mistletoe lectin I, ML I), inevitable to the family of RIPs, was identified in the late 1980s as the main pharmacologically-active ingredient of mistletoe (*Viscum album*) extract and is largely responsible for its toxicity (Krauspenhaar et al. 1999). Viscumin toxicity is high. The LD_{50} for mice with intraúeritoneal administration is 2 µg/kg and is therefore comparable to ricin toxicity (Patocka et al. 2004) and acts by the same mechanism. Viscumin has a concentration-dependent activity profile unique to plant AB-toxins. It starts with lectin-dependent mitogenicity and then covers toxicity and cell agglutination, associated with shifts in the monomer/dimer equilibrium (Jiménez et al. 2006). When viscumin binds to its target cell, protein synthesis in that cell is interrupted as a result of the A-chain's enzymatic activity, like a ricin. This interruption induces a cellular stress response, which triggers the release of cytokines by the target cell and, at high viscumin concentrations, apoptosis of the cell (Thies et al., 2005). Viscumin belongs to a group of selected substances, according to the Centers for Disease Control and Prevention, or the control of trade in dual-use products in the European Union (Duracova et al., 2018).

Table II. Published toxicity data for ricin

Organism	Test Type [a]	Route	Report Dose	Reference
mouse	LD_{50}	intraperitoneal	2 µg/kg	Creppy et al., 1980
mouse	LD_{50}	intravenous	2.2 µg/kg	NTIS, 2017
mouse	LD_{50}	subcutaneous	2.21 µg/kg	NTIS, 2017
mouse	LD_{50}	unreported	6.0 µg/kg	Gürtler and Horstmann, 1973
mouse	LDLo	oral	30 mg/kg	Ishiguro et al., 1983
rat	LD_{50}	intraperitoneal	1.5 µg/kg	Creppy et al., 1980
rat	LD_{50}	unreported	5 µg/kg	NTIS, 2017
rat	LD_{50}	parenteral	0.336 µg/kg	Strocchi et al., 1979
rat	LD_{50}	unreported	4 µg/kg	NTIS, 2017
rat	LDLo	oral	30 mg/kg	Ishiguro et al., 1983
guinea pig	LD_{50}	unreported	0.8 µg/kg	NTIS, 2017
guinea pig	LDLo	intraperitoneal	0.02 µg/kg	Mosher et al., 1964
rabbit	LD_{50}	intratracheal	0.5 µg/kg	NTIS, 2017
rabbit	LD_{50}	intravenous	0.54 µg/kg	Christiansen et al., 1994
rabbit	LD_{50}	unreported	0.1 µg/kg	NTIS, 2017
rabbit	LDLo	oral	500 µg/kg	PCOC, 1966
cat	LD_{50}	intratracheal	5 µg/kg	NTIS, 2017
cat	LD_{50}	unreported	0.2 µg/kg	NTIS, 2017
dog	LD_{50}	intramuscular	0.6 µg/kg	Marhold, 1986
dog	LD_{50}	intratracheal	5 µg/kg	NTIS, 2017
dog	LD_{50}	unreported	0.6 µg/kg	NTIS, 2017
dog	LDLo	unreported	1.6 µg/kg	Fodstad et al. 1979
dog	LDLo	intravenous	1.6 µg/kg	Fodstad et al. 1979
sheep	LD_{50}	unreported	0.8 µg/kg	NTIS, 2017
human	LDLo	oral	2 mg/kg	PCOC, 1966
human	LDLo	oral	0,3 mg/kg	NTIS, 2017
human	LDLo	oral	0,9 mg/kg	Kopferschmitt et al., 1983

[a] LD_{50} = Acute Lethal Dose, LDLo = Lethal Dose Low

VOLKENSIN

Volkensin is a lectin from *Adena volkensii* (the kilyambiti plant) that is comparable in toxicity to ricin and that acts by the same mechanism (Wu and Sun, 2012). The toxin is a glycoprotein (mol wt. 62,000, neutral sugar content 5.74 %) consisting of an A subunit (mol wt. 29,000) and of a B subunit (Mr 36,000) linked by disulfide and noncovalent bond(s) (Stirpe et al., 1985). The plant is a relatively unattractive and toxic succulent plant found in Africa that appears to be of little interest. However, it has proven useful as a research reagent in neurology because of its ability to be taken up and transported by certain types of nerve (Wiley and Stirpe, 1987).

Although volkensin belongs to the same group of poisonous proteins as abrin or ricin, and its toxicity is comparable (LD_{50}, intraperitoneal mice 1.38 µg/kg) with ricin and abrin (Barbieri et al. 1984), it seems to differ in some respects (Wiley and Stirpe, 1988). If it is injected in the rat dorsal hippocampus, volkensin is taken up

by nerve terminals in the injected area of the brain and retrogradely transported to the cell bodies originating the projection, which are killed by the toxin (Contestabile et al., 1990). Volkensin-induced selective motoneuron death in the adult rat can be a useful experimental model for degenerative motoneuron disease (Nogradi and Vrbova, 1992).

Experimental lesions and quantitative autoradiography were used to investigate the cellular distribution of neurotransmitter receptors in rats. Lesions were produced by intracortical injections of either volkensin or ricin. However, only volkensin is retrogradely transported and volkensin treatment causes significant loss of contralateral cortical pyramidal neurones. (Chessell et al., 1997).

MODECCIN

Modeccin is a lectin from the roots of *Adenia digitata* an African succulent plant (Stirpe etal., 1977) that is comparable in toxicity to ricin (Olsnes et al. 1982) and acts by the same mechanism (Refsnes et al. 1977, Olsnes et al. 1978). The plant does not seem to have any significant uses, such as food or medicine and so is not available in quantities comparable to abrin, let alone ricin. However, the seed does seem to be readily available. The subunits were isolated of modeccin (subsequently referred to as modeccin 4B), purified from the roots of Adenia digitata by affinity chromatography on Sepharose 4B (Gasperi-Campani et al. 1978). They are an A subunit (mol.wt. 26 000), which inhibits protein synthesis, and a B subunit (mol.wt. 31 000), which binds to cells. Both subunits, as well as intact modeccin, gave single bands on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, but showed some heterogenity on isoelectric focusing and on polyacrylamide-gel electrophoresis at pH 9.5.

A second form of modeccin, not retained by Sepharose 4B, was purified by affinity chromatography on acid-treated Sepharose 6B: this form is subsequently termed modeccin 6B. Modeccin 6B has a molecular weight indistinguishable from that of modeccin 4B, and consists of two subunits of mol.wts. 27 000 and 31 000, joined by a disulphide bond. The subunits were not isolated because of their high insolubility in the absence of sodium dodecyl sulphate. As compared with modeccin 4B, modeccin 6B is slightly less toxic to animals, does not agglutinate erythrocytes, and is a more potent inhibitor of protein synthesis in a lysate of rabbit reticulocytes, giving 50% inhibition at the concentration of 0.31 mg/ml (Barbieri et al. 1980).

CONCLUSIONS

Ribosome-inactivating proteins (RIPs) abrin, ricin, viscumin, volkensin and modeccin are very potent toxins derived from plants. They are extremely toxic for all warm-blooded animals including human and represent potential biological warfare toxin and easy exploitable means for terroristic attack. Especially ricin for its easy availability is alluded as acute terrorist danger very often lately.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ACKNOWLEDGMENTS

This work was supported by the long-term organization development plan (University Hospital, Hradec Kralove, Czech Republic).

REFERENCES

1. Anderson PD. Bioterrorism: toxins as weapons. J Pharm Pract. 2012;25(2):121-129.
2. Anonymous. AMA Arch Ind Health. 1955;12:468.
3. Audi J, Belson M, Patel M, Schier J, Osterloh J. Ricin poisoning: a comprehensive review. JAMA. 2005;294(18):2342-2351.
4. Balint GA. Ricin: The toxic protein of castor oil seeds. Toxicology. 1974;2:77–102.
5. Barbieri L, Baltelli M, Stirpe F. Ribosomes-inactivating proteins from plants. Biochemica Biophysica Acta. 1993;1154:237–282.

6. Barbieri L, Falasca AI, Stirpe F. Volkensin, the toxin of Adenia volkensi (kilyambiti plant). FEBS Lett. 1984;171(2):277-279.
7. Barbieri L, Zamboni M, Montanaro L, Sperti S, Stirpe F. Purification and properties of different forms of modeccin, the toxin of Adenia digitata. Separation of subunits with inhibitory and lectin activity. Biochem J. 1980;185(1):203-210.
8. Berdy J. CRC Handbook of Antibiotic Compounds, Vol.1 , Boca Raton, FL, CRC Press, 1982;8(1):107.
9. Bradberry SM, Dickers KJ, Rice P, Griffiths GD, Vale JA. Ricin poisoning. Toxicol Rev. 2003;22(1):65-70.
10. Chaturvedi K, Jadhav SE, Bhutia YD, Kumar O, Kaul RK, Shrivastava N. Purification and dose-dependent toxicity study of abrin in swiss albino male mice. Cell Mol Biol (Noisy-le-grand). 2015;61(5):36-44.
11. Chen YL, Chow LP, Tsugita A, Lin JY. The Complete Primary Structure of Abrin-a B Chain. FEBS Lett. 1992;309:115-118.
12. Chessell IP, Pearson RC, Heath PR, Bown DM, Francis PT. Selective loss of cholinergic receptors following unilateral intracortical injection of volkensin. Exp Neurol. 1997;147(1):183-191.
13. Cho H, Jaworski J. A portable and chromogenic enzyme-based sensor for detection of abrin poisoning. Biosens Bioelectron. 2014;54:667-673.
14. Christiansen VJ, Hsu CH, Dormer KJ, Robinson CP. The cardiovascular effects of ricin in rabbits. Pharmacol Toxicol. 1994;74(3):148-152.
15. Contestabile A, Fasolo A, Virgili M, Migani P, Villani L, Stirpe F. Anatomical and neurochemical evidence for suicide transport of a toxic lectin, volkensin, injected in the rat dorsal hippocampus. Brain Res. 1990;537(1-2):279-286.
16. Creppy EE, Lugnier AA, Dirheimer G. Isolation and properties of two toxic tryptic peptides from ricin, the toxin of Ricinus communis L. (castor bean) seeds. Toxicon. 1980;18(5-6):649-660.
17. Crompton R, Gall D. Georgi Markov--death in a pellet. Med Leg J. 1980;48(2):51-62.
18. Cummings RD, L. Schnaar R. R-Type Lectins. In: Varki A, Cummings RD, Esko JD, Stanley P, Hart GW, Aebi M, Darvill AG, Kinoshita T, Packer NH, Prestegard JH, Schnaar RL, Seeberger PH, editors. Essentials of Glycobiology, 3rd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2015-2017. Chapter 31, 2017.
19. Das MK, Sharma RS, Mishra V. Induction of apoptosis by ribosome-inactivating proteins: importance of N-glycosidase activity. Appl Biochem Biotechnol. 2012;166(6):1552-1561.
20. Diac M, Matei MC, Manea C, Schiopu C, Iliescu DB, Furnica C, Chistol RO, Knieling A. Intoxication with Ricin-Biochemical Weapon. Revistade Chimie. 2017;68(6):1329-1332.
21. Duracova M, Klimentova J, Fucikova A, Dresler J. Proteomic Methods of Detection and Quantification of Protein Toxins. Toxins (Basel). 2018, 10(3). pii: E99.
22. Fodstad O, Johannessen JV, Schjerven L, Pihl A. Toxicity of abrin and ricin in mice and dogs. J Toxicol Environ Health. 1979;5(6):1073-1084.
23. From S, Płusa T. [Today's threat of ricin toxin]. Pol Merkur Lekarski. 2015;39(231):162-164. Article in Polish.
24. Garber EA, Venkateswaran KV, O'Brien TW. Simultaneous multiplex detection and confirmation of the proteinaceous toxins abrin, ricin, botulinum toxins, and Staphylococcus enterotoxins a, B, and C in food. J Agric Food Chem. 2010;58(11):6600-6607.
25. Gasperi-Campani A, Barbieri L, Lorenzoni E, Montanaro L, Sperti S, Bonetti E, Stirpe F. Modeccin, the toxin of Adenia digitata. Purification, toxicity and inhibition of protein synthesis in vitro. Biochem J. 1978;174(2):491-496.
26. Godal A, Olsnes S, Pihl A. Radioimmunoassays of abrin and ricin in blood. J Toxicol Environ Health. 1981;8(3):409-417
27. Griffiths GD, Lindsay CD, Allenby AC, Bailey SC, Scawin JW, Rice P, Upshall DG. Protection against inhalation toxicity of ricin and abrin by immunisation. Hum Exp Toxicol. 1995;14(2):155-164.
28. Gürtler LG, Horstmann HJ. Subunits of toxin and agglutinin of Ricinus communis. Biochim Biophys Acta. 1973;295(2):582-594.
29. Ishiguro M, Mitarai M, Harada H, Sekine I, Nishimori I, Kikutani M. Biochemical studies on oral toxicity of ricin. I. Ricin administered orally can impair sugar absorption by rat small intestine. Chem Pharm Bull (Tokyo). 1983;31(9):3222-3227.
30. Jiménez M, André S, Siebert HC, Gabius HJ, Solís D. AB-type lectin (toxin/agglutinin) from mistletoe: differences in affinity of the two galactoside-binding Trp/Tyr-sites and regulation of their functionality by monomer/dimer equilibrium. Glycobiology. 2006;16(10):926-937.

31. Kent K. J. Ricin. In Handbook of Bioterrorism and Disaster Medicine Springer, Boston, MA. 2006, 143-146.
32. Kimura M, Sumizawa T, Funatsu G. The complete amino acid sequences of the B-chains of abrin-a and abrin-b, toxic proteins from the seeds of Abrus precatorius. Biosci Biotechnol Biochem. 1993;57:166-169.
33. Kopferschmitt J, Flesch F, Lugnier A, Sauder P, Jaeger A, Mantz JM. Acute voluntary intoxication by ricin. Hum Toxicol. 1983;2(2):239-242.
34. Kozlov IV, Sudarkina OI, Kurmanova AG. [Ribosome-inactivating lectins from plants]. Mol Biol (Mosk). 2006;40(4):711-723. Article in Russian
35. Krauspenhaar R., S. Eschenburg, M. Perbandt, V. Kornilov, N. Konareva, I. Mikailova, S. Stoeva, R. Wacker, T. Maier, T. Singh, A. Mikhailov, W. Voelter, C. Betzel: Crystal structure of mistletoe lectin I from Viscum album. Biochem. Biophys. Res. Commun. 1999;257:418–424.
36. Kuca K, Pohanka M. Chemical warfare agents. EXS. 2010;100:543-558.
37. Kumar MS, Karande AA. A monoclonal antibody to an abrin chimera recognizing a unique epitope on abrin A-chain confers protection from abrin-induced lethality. Hum Vaccin Immunother. 2016;12(1):124-131.
38. Larsson SL, Sloma MS, Nygard O. Conformational changes in the structure of domains II and V of 28S rRNA in ribosomes treated with the translational inhibitors ricin or alpha-sarcin. Biochem. Biophys. Acta 2002;1577:53–62.
39. Lin JY, Shaw YS, Tung TC. Studies on the active principle from Abrus precatorius L. leguminosae seed kernels. Toxicon. 1971;9(2):97-101.
40. Liu S, Tong Z, Mu X, Liu B, Du B, Liu Z, Gao C. Detection of Abrin by Electrochemiluminescence Biosensor Based on Screen Printed Electrode. Sensors (Basel). 2018;18(2). pii: E357.
41. Lopez Nunez OF, Pizon AF, Tamama K. Ricin Poisoning after Oral Ingestion of Castor Beans: A Case Report and Review of the Literature and Laboratory Testing. J Emerg Med. 2017;53(5):e67-e71.
42. Lord JM, Roberts LM, Robertus JD. Ricin: structure, mode of action, and some current applications. FASEB J 1994;8:201-208.
43. Madsen JM. Toxins as weapons of mass destruction. A comparison and contrast with biological-warfare and chemical-warfare agents. Clin Lab Med. 2001;21(3):593-605.
44. Manske JM, Buchsbaum DJ, Vallera DA. The role of ricin B chain in the intracellular trafficking of anti-CD5 immunotoxins. J. Immunol. 1989;142:1755–1766.
45. Merck Index. Encyclopedia of Chemicals, Drugs, and Biologicals, 11th ed., Rahway, NJ 07065, Merck & Co., Inc. 1989, Vol. 11, Pg. 2.
46. Mosher HS, Fuhrman FA, Buvhwald HD, Fischer HG. Tarichatoxin - tetrodotoxin: a potent neurotoxin. Science. 1964;144(3622):1100-1110.
47. Nogradi A, Vrbova G. The use of a neurotoxic lectin, volkensin, to induce loss of identified motoneuron pools. Neuroscience. 1992;50(4):975-986.
48. NTIS, National Technical International Service, Vol. PB158-508. 2017. Available on https://www.ntis.gov/
49. Olsnes S, Pihl A. Kinetics of binding of the toxic lectins abrin and ricin to surface receptors of human cells. J Biol Chem 1976;251:3977-3984.
50. Olsnes S, Refsnes K, Pihl A. Mechanism of action of the toxic lectins abrin and ricin. Nature 1974;249:627–631.
51. Olsnes S, Sandvig K, Eiklid K, Pihl A. Properties and action mechanism of the toxic lectin modeccin: interaction with cell lines resistant to modeccin, abrin, and ricin. J Supramol Struct. 1978;9:15-25.
52. Olsnes S. The history of ricin, abrin and related toxins. Toxicon. 2004;44:361–370.
53. Papaloucas M, Papaloucas C, Stergioulas A. Ricin and the assassination of Georgi Markov. Pak J Biol Sci. 2008;11(19):2370-2371.
54. Patocka J. Abrin and ricin - two dangerous poisonous proteins. ASA Newsletter. 2001;85:205-208.
55. Patocka J, Bajgar J. Toxicology of perfluoroisobutene. ASA Newsletter. 1998, 16-18.
56. Patocka J, Hon Z, Streda L, Kuca K, Jun D. Biohazards of protein biotoxins. Defence Science Journal. 2007;57(6):825.
57. Patocka J, Streda L. Plant toxic proteins and their current significance for warfare and medicine. J Appl Biomed. 2003;1:141-147.
58. Patocka J, Streda L. Protein biotoxins of military significance. Acta Medica (Hradec Kralove). 2006;49(1):3-11.
59. Patocka and coll. Military Toxicology. Grada Publishing, Avicenum, Praha, 2004.178 pp. ISBN 80-247-0608-3 Article in Czech
60. PCOC, Pesticide Chemicals Official Compendium, Association of the American Pesticide Control Officials Inc., 1966, 963.

61. Pearson GS. Iraq biological weapons programme. In: Pearson GS, Whitman J. (eds.) The UNSCOM saga: chemical and biological weapons non-proliferation. London: Macmillan Press; 1999, p. 126.
62. Pittman PR, Reisler RB, Lindsey CY, Güereña F, Rivard R, Clizbe DP, Chambers M, Norris S, Smith LA. Safety and immunogenicity of ricin vaccine, RVEc™, in a Phase 1 clinical trial. Vaccine. 2015;33(51):7299-7306.
63. Poli MA, Rivera VR, Hewetson JF, Merril GA. Detection of ricin by colorimetric and chemiluminescence ELISA. Toxicon. 1994;32:1371–1377.
64. Rauber A, Heard J. Castor bean toxicity re-examined: A new perspective. Vet Hum Toxicol. 1985;27:498–502.
65. Refsnes K, Haylett T, Sandvig K, Olsnes S. Modeccin--a plant toxin inhibiting protein synthesis. Biochem Biophys Res Commun. 1977;79(4):1176-1183.
66. Rust A, Partridge LJ, Davletov B, Hautbergue GM. The Use of Plant-Derived Ribosome Inactivating Proteins in Immunotoxin Development: Past, Present and Future Generations. Toxins (Basel). 2017;9(11). pii: E344.
67. Sharon N, Lis H. Cell-agglutinating and sugar-specific proteins. Science. 1972;177:949–959.
68. Shi WW, Mak AN, Wong KB, Shaw PC. Structures and Ribosomal Interaction of Ribosome-Inactivating Proteins. Molecules. 2016;21(11). pii: E1588
69. Soler-Rodriguez AM, Uhr JW, Richardson J, Vitetta ES. The toxicity of chemically deglycosylated ricin A-chain in mice. Int J Immunopharmacol. 1992;14(2):281-291.
70. Spivak L, Hendrickson RG. Ricin. Crit Care Clin. 2005;21(4):815-824, viii.
71. Stillmark R. Ueber Ricen. Arbeiten des Pharmacologischen Institutes zu Dorpat, iii, 1889. Cited in: Flexner, J. The histological changes produced by ricin and abrin intoxications. J Exp Med. 1897;2:197–216.
72. Stirpe F, Barbieri L, Abbondanza A, Falasca AI, Brown AN, Sandvig K, Olsnes S, Pihl A. Properties of volkensin, a toxic lectin from Adenia volkensii. J Biol Chem. 1985;260(27):14589-14595.
73. Stirpe F, Battelli MG. Ribosome-inactivating proteins: Progress and problems. Cell. Mol. Life Sci. 2006;63:1850–1866.
74. Stirpe F, Gasperi-Campani A, Barbieri L, Lorenzoni E, Montanaro L, Sperti S, Bonetti E. Inhibition of protein synthesis by modeccin, the toxin of Modecca digitata. FEBS Lett. 1977;85(1):65-67.
75. Strocchi P, Novello F, Montanaro N, Stirpe F. Effect of intraventricularly injected ricin on protein synthesis in rat brain. Neurochem Res. 1979;4(2):259-268.
76. Sundan A, Sandvig K, Olsnes S: Preparation and properties of a hybrid toxin of modeccin A-chain and ricin B-chain. Biochim. Biophys. Acta. 1983;76:296–302.
77. Thies A, Nugel D, Pfüller U, Moll I, Schumacher U. Influence of mistletoe lectins and cytokines induced by them on cell proliferation of human melanoma cells in vitro. Toxicology. 2005;207(1):105-116.
78. Tung TC, Lin JY, Hsu CT, Some aspects on mechanism of anti-cancer activity of abrin. Toxicon. 1975;13:127.128.
79. Wang J, Gao S, Xin W, Kang L, Xu N, Zhang T, Liu W, Wang J. A novel recombinant vaccine protecting mice against abrin intoxication. Hum Vaccin Immunother. 2015;11(6):1361-1367.
80. Wiley RG, Stirpe F. Modeccin and volkensin but not abrin are effective suicide transport agents in rat CNS. Brain Res. 1988;438(1-2):145-154.
81. Wiley RG, Stirpe F. Neuronotoxicity of axonally transported toxic lectins, abrin, modeccin and volkensin in rat peripheral nervous system. Neuropathol Appl Neurobiol. 1987;13(1):39-53.
82. Wu W, Sun R. Toxicological studies on plant proteins: a review. J Appl Toxicol. 2012;32(6):377-386.
83. Xu C, Li X, Liu G, Xu C, Xia C, Wu L, Zhang H, Yang W. Development of ELISA and Colloidal Gold-PAb Conjugate-Based Immunochromatographic Assay for Detection of Abrin-a. Monoclon Antib Immunodiagn Immunother. 2015;34(5):341-345.
84. Zhang T, Kang L, Gao S, Yang H, Xin W, Wang J, Guo M, Wang J. Truncated abrin A chain expressed in Escherichia coli: a promising vaccine candidate. Hum Vaccin Immunother. 2014;10(9):2648-2655.