Intoxication with Ricin - Biochemical Weapon

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Ricin, a toxic glycoprotein found in the seeds of castor oil plant, is capable of irreversible cellular adhesion and inhibition of protein synthesis. The authors performed an up to date review concerning the chemical structure, mechanism of action, poisoning symptoms and treatment, and potential uses of ricin as a biochemical weapon. Castor oil plant is easy to cultivate and harvest worldwide and, except the United States of America, cultures and processing plants are not supervised. Ricin extraction does not require laborious and costly technique and it is undetectable once in the body (except for urine in case of ricin ingestion). Poisoning generates nonspecific symptoms and is potentially fatal with no antidote or specific treatment available. Forensic specialists must be aware of symptoms and post-mortem findings in order to make a correct diagnosis of ricin poisoning.

Keywords: ricin, glycoprotein, poisoning, fatal, protein synthesis

Castor oil plant (*Ricinus communis)* belongs to *Euphorbiaceae* family, its Latin name being derived from the word *ricinus* meaning tick, suggestive for the aspect of its seeds. It is also named *Christuspalme* (the palm of Christ), *Kreutzbaum* (cross three) or "Wunderbaum" (wonder tree) because of the shape of its leaves. Castor oil plant originates from Africa but it is cultivated worldwide, including Romania, as it is largely used in industry. Castor oil plant produces 8 - 20 mm long and 4 - 12 mm wide seeds, depending on the variety. These seeds contain 46-53% oil, composed of glycerides of different acids such as ricinoleic and iso-ricinoleic [1, 2]. The oil is used in medicine (purgative action of the two acids), manufacture of paints, soaps, varnishes, cosmetics, lubricants and biofuels among others. Castor oil also contains an alkaloid called ricinine ($C_8H_8N_2O_2$ - 1,2-Dihydro-4-methoxy-1-methyl-2-oxo-3-pyridinecarbonitrile), enzymes and vitamin E. A special feature of this oil is represented by the presence of a hydroxyl group attached to the carbon chain and responsible for its increased viscosity and alcohol solubility al relatively low temperatures [3, 4].

Pressed oil has a high protein content and hence it is used as an organic fertilizer. Following extraction of castor oil, ricin, a lethal phytotoxin is left in the press-cake. It can be destroyed by heat treatment (80°C for 10 minutes or 50°C for an hour) for press-cakes to be used in livestock feeding. Cases of animal intoxication have been reported because of insufficient heat treatment [5].

Ricin is a non-liposoluble toxic glycoprotein composed of two polypeptide chains joined by a disulfide bond and belongs to the type II group of ribosome inactivating proteins (type II RIP). The toxin is endocytosed by the cells and inactivates ribosomal ribonucleic acid (rRNA) thus causing cell death [6].

Experimental part

Material and methods

The authors performed an up to date review concerning the chemical structure, mechanism of action, poisoning symptoms and treatment, and potential uses as a biochemical weapon of toxins derived from castor oil plant seeds.

The experimental procedures were carried out in accordance with the mandatory principles of the Ethical Committee of the Grigore T. Popa University of Medicine and Pharmacy Iasi [7, 8].

Chemical structure and mechanism of action

Chemical structure

Ricin is a glycoprotein characterized by the presence of two polypeptide chains: one capable of inhibiting protein synthesis and one with lectin (carbohydrate-binding) properties. Chain A (ricin toxin A - RTA) is composed of 267 amino acids, weights 30-32 kDa and is linked by a disulfide bond to chain B (ricin toxin B - RTB) composed of 262 amino acids and weighting 32-34 kDa. RTA is a member of the N-glycosidase enzymatic class and can inhibit protein synthesis by hydrolysing the N-glycosidic bond between adenine and ribose in rRNA. RTB is a lectin with a preference for galactose binding. There are four disulfide bridges and two domains in its structure, each with four subdomains (λ, α, β and γ). Subdomains 1α and 2γ have the ability to bind to galactose. Ricin has two glycosylation zones in the RTB and one or two in the RTA. Two ricin isoforms have been described, isoform D and E, with the toxicity of ricin D being higher than ricin E. The different cytotoxicity could be due to differences in the structure of RTB. Ricin E has a lower galactose binding capacity thus a decreased internalisation in the cell [9-11].

In addition to the two isotoxins, an agglutinin with affinity for terminal β-D-galactosyl residues is also present in castor oil plant seeds. This agglutinin has two A chains and two B chains in its structure. For the agglutinin, there are also 2 known isoforms with different weight and amino acid composition responsible for different affinity [12].

Variations are influenced by the variety and country of origin of the plant and other lectins may also be present.

Ricin is soluble in saline solutions, insoluble in lipids and ethanol. It can present in a crystalized form or as a white powder, with no odour or taste.

Mechanism of action

RTB mediates ricin attachment to cell's membrane followed by receptor-mediated endocytosis. Once

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internalized, ricin can follow 3 pathways: degradation by lysosomes, recirculation to the cell surface or transportation to the Golgi apparatus (4-6%). From the Golgi apparatus ricin arrives to the endoplasmic reticulum (ER) where an enzyme, disulfide isomerase, separates RTA from RTB. RTA is further transported to the cytosol in order to be degraded by the proteasomes. RTA presents a limited number of lysine residues thus decreasing the possibility of its degradation and increasing the chance that it arrives to the ribosomes [13].

The amino acids responsible for N-glycosidase activity of the of the RTA are glutamic acid at position 177 and arginine at position 180. Two tyrosine residues were also identified at positions 80 and 123, between whom RNA would fit. The breakdown of the bond between the nitrogen N-9 of adenine and C-1' carbon of the rRNA ribose would be facilitated by the protonation of N-3 nitrogen by the arginine at position 180. Ribose is thus distorted to the oxocarbenium-ion by the departure of the purine base. The carboxylate from the glutamate at position 177 binds to the oxocarbenium-ion and stabilizes it due to the negative charge. Protonation of the N-3 nitrogen of adenine increases arginine base character allowing it to capture a hydrogen atom from a water molecule in order to form a hydroxyl ion which finally attacks the carbon C-1 'of the ribose and neutralizes its positive charge. The inhibitory action of RTA on protein synthesis is catalytic and irreversible, 1500 ribosomes being affected per minute [14, 15].

Ricin also has lipase activity and apoptotic action mediated by other amino-acids than those involved in the inhibition of protein synthesis.

Toxicology

Individuals exposed to ricin exhibit different symptoms depending on the route of administration. Initially, symptoms may mimic a typical gastroenteritis or respiratory disease. For this reason, one should pay attention to epidemiological indicators suggesting the possibility of a biological attack (unusual number of patients accusing similar symptoms).

Inhalation

Inhalation, as expected during a biological attack, causes cell damage mainly to the lungs, a different effect than if the toxin is injected. What's more, for one equivalent dose of toxin, more antibodies should be injected to protect an individual from the inhalation than an intravenous injection of the toxin.

Firsts symptoms to occur are represented by cough, fever, nausea, difficulty in breathing (4-8 h) finally evolving towards acute respiratory distress syndrome (18-24 h). Death generally occurs 36-72 hafter exposure. Workers exposed to the dust of castor oil plants have developed allergic reactions such as nasal and throat congestion, eye irritation, urticaria and asthma [16].

Experimental studies on rats exposed to ricin aerosols revealed apoptosis and epithelial necrosis in respiratory tract epithelium and alveoli together with intra-alveolar oedema [17]. Wilhelmsen and Pitt conducted a study on five primates who received aerosols with a total dose of 20.95-41.8 micrograms of ricin/kg of body weight. Three of them died at 36, 40 and 48 h after the exposure and the other two were sacrificed at 48 h because of severe respiratory distress. The autopsy revealed necrosis and oedema in the airways (especially terminally and respiratory bronchioles) and alveoli as well as fibrin deposits in the alveoli together with inflammation and necrosis of the lymph nodes draining the tracheobronchial tree [18].

Oral ingestion

Symptoms from oral ingestion of the purified toxin are similar to those presented by mastication and ingestion of castor seeds.

A mid-level exposure can cause nausea, vomiting, diarrhoea and/or abdominal pain. Typical symptoms of a gastrointestinal disease occur 1 to 4 hours after ingestion. In case of medium to severe exposure, gastrointestinal symptoms are persistent and include vomiting, profuse diarrhoea associated to hematemesis and melena due to mucosa haemorrhage. Dysphagia can also occur secondary to inflammation. Severe dehydration can lead to hypovolemic shock. Finally, liver and kidney failure installs leading to death 3-5 days after exposure. Supportive treatment including repeated gastric lavage, administration of active charcoal, electrolytic and volemic compensation, vasopressor drugs may succeed if initiated quickly [19].

Autopsy of poisoned humans and animals revealed superficial erosions in the stomach, atrophy and inflammation of intestinal villi, liver, spleen, kidney, thymus, lung and heart injuries [20, 21].

Skin exposure

In the case of skin exposure, the amount absorbed is insignificant. The toxin can be can be absorbed dermally if boosted with a strong solvent or in case of prior lesions leading to dermal exposure. Symptoms of dermal absorption depend on the type of solvent and the period of exposure (strong irritation with pruritus, burning sensation, vesicles or strong allergic reaction). This type of exposure is incapable of causing high toxicity [22].

In case of dermal exposure, skin decontamination should be performed. The person should wash its hands with soap and water and avoid taking them to the mouth.

Eye exposure

Eye exposure to ricin powder leads to mild to moderate symptoms including lacrimation, inflammation, conjunctivitis. Prolonged exposure or high dose may lead to pseudomembranous conjunctivitis, retinae haemorrhage, optic nerve involvement with blindness, systemic toxicity and death [23].

Injection

Only one human test has been performed in order to evaluate low doses of intravenous ricin as a chemotherapy agent. Injected ricin caused weakness within 5 hours, fever and vomiting at 24 hours, followed by collapse and failure of various organs, causing the death of the individual in the third day. The victim initially presented severe local necrosis followed by regional necrosis in the lymph nodes, and finally of major organs [24].

Otherwise, three other cases of parenteral administration of castor seeds extract are known. A chemist self-administered two intramuscular doses (150 mg of ricin obtained from aqueous extraction of seeds) in an autolytic attempt. After a latency of 10 hours, the man presented erythema at the site of administration (the anterior compartment of the right thigh and right gluteal region), lymph node swelling, headache, fever, chills. The fever persisted for 8 days but the patient recovered with no complications [25]. A young man (20-year-old) self-administered subcutaneously an undetermined amount of extract of castor oil plant seeds also in an autolytic attempt. He was admitted to hospital 36 h later with nausea, dizziness, headache, chest tightness, abdominal pain, myalgia in the extremities, tachycardia, hypotension, anuria, metabolic acidosis, ecchymosis and oedema at the site of administration. Multiple organ dysfunction syndrome (MODS) occurred and the man died 18 hours

after admission [26]. Another case is the one of a 53-years-old man who self-administered also in an autolytic attempt the extract of 13 chewed castor oil plant seeds. Necrosis was observed at the site of administration and was surgically excised. The patient was discharged 3 months after the suicidal attempt [27].

Two known cases involve the use of ricin as a biological weapon by the Komitet gosudarstvennoy bezopasnosti (KGB) in august 1978. A Bulgarian dissident living in exile in Paris, Vladimir Kostov, felt a puncture in the back while in the subway. He was hospitalized for 12 days with fever as the only sign. A small metal ball with 2 holes was identified at the puncture site. Kostov survived the assassination attempt. Later that year, the Bulgarian British Broadcasting Corporation (BBC) journalist and dissident Georgi Markov was assassinated by a KGB agent who administered ricin using an umbrella modified in order to contain a shooting device with a damping cylinder. The killer shot from 1.5-2 meters a small bullet containing approximately 500 micrograms of ricin. Markov recalls feeling a puncture in the back of the right thigh and a man carrying an umbrella asking for his forgiveness. The next day, he was admitted to the hospital with fever, vomiting, and mild leucocytosis (10600/mm^3). 4 days after he was shot, Markov developed severe leucocytosis (33200/mm^3) and died the same day of cardiac arrest. At the autopsy, a small metal ball similar to the one discovered in Kostov was identified in his right thigh. Haemorrhagic infiltrates were found over the heart and intestines together with pulmonary oedema, and lymph node necrosis. Ricin was never actually identified in these two cases, but the Chemical and Biological Defence Establishment of the United Kingdom indicated ricin as the lethal toxin based on histopathological observations and information obtained by intelligence services that were aware of the existence of military programs investigating the use of ricin as a biological weapon in countries member of the Warsaw Pact [1, 28].

Several attempts to use ricin as a biological weapon in the United States (US) are known. In 2003 ricin was discovered in two letters mailed to the White House and senator Bill Frist [29]. In 2013, the martial arts instructor Everett Dutschke sent 3 letters tested positive for ricin to senator Roger Wicker, president Obama and judge Sadie Holland. In the US, ricin is classified as an extremely dangerous substance in class A of risk to being used as a biological weapon [30, 31].

Lethal dose

Ricin is seven times more deadly than the poison of a cobra and six thousand times more deadly than cyanide [32]. Lethal dose for an adult varies according to administration pathway.

If inhaled or injected, a single dose of 3 to 5 micrograms per kilogram of body weight is lethal (210 to 350 micrograms for a 70-kg individual) according to some authors. Thus, a dose of 500 micrograms, about the size of a pinhead, is sufficient to kill an average adult [24]. The Chemical and Biological Defence Establishment of the United Kingdom government indicate that the lethal dose of injected ricin is 1-10 micrograms/kg of body weight [33].

Because ricin is not well absorbed well in the gastrointestinal tract, the lethal dose per ingestion must be greater. It is estimated that 1 milligram of ricin ingested per 9.6 kg of body weight can be lethal. There are discrepancies in the minimum number of seeds that can be lethal to man because of the different toxicity of plants from various parts of the world. Usually, if the intake exceeds 1 seed in children or 8 in adults, the dose is considered potentially lethal.

Seed's shell cannot be digested so accidental poisoning is very rare as they have to be chewed. Small, progressive doses, can lead to resistance, experimental animals resisting even to administration of 800 lethal doses [5]. No antidote exists and death occurs with 4 to 32 hours depending on the administration pathway (ingested, inhaled, injected, skin contact) [34].

Experimental use as a chemotherapeutic drug

The cytotoxic activity of ricin is used in experimental therapy of cancer by binding RTA to a monoclonal antibody. One clinical trial involved intravenous administration of 4.5-23 micrograms of ricin/m^2 of body surface area for two weeks. Doses exceeding 18 micrograms/m^2 of body surface area generated fever and influenza-like symptoms after 4-6 h from administration. The symptoms disappeared 1-2 days later. Other clinical trials indicate as side effect increased vascular permeability with fluid extravasation and oedema due to the direct action of RTA on endothelial cells [35, 36].

Diagnosis

Paraclinical tests are as nonspecific as the symptoms and may reveal severe sepsis. The high levels of hepatic enzymes (transaminases), renal tests, inflammatory markers and leucocytosis may mislead the diagnosis towards a severe infection. Haemolytic anaemia together with hydro-electrolytic and acid-base imbalance should suggest ricin poisoning rather than infection. No specific tests exist to early detect ricin in the body, it is excreted through urine 3 days after ingestion. For contaminated sites, the liberation of ricin aerosols or its presence on surfaces can be detected with military devices not available for civil personnel (hospitals).

The differential diagnosis is broad as ricin poisoning initially mimics common diseases (asthma, food poisoning) or the toxicity of other microbial agents such as *Corynebacterium diphtheriae*, *Bacillus anthracis*, *Yersinia pestis* [37].

Treatment. Antidote

Up to date, no antidote is available for ricin poisoning. The treatment is mostly supportive ad involves decontamination (gastric lavage, charcoal), volemic support (crystalloid solutions), vasopressor drugs and blood transfusions. Despite maximal support, most patients die because of MODS. The highest survival rate is registered in case of ingestion but with potential permanent organ dysfunction.

Anti-Ricin toxin A and B antibodies were tested in rabbits and mice and displayed some neutralization action. They could mostly be used for detection of RTA and RTB in enzyme-linked immunosorbent assay (ELISA) and western blotting [38].

Soligenix® presented a paper *Serum Antibody Profiling following Vaccination Reveals a Correlate of Immunity to Ricin Toxin* on April 25th 2017 that analysed the efficiency of the company's proprietary vaccine candidate for the prevention of exposure to ricin toxin, RiVax™, that utilizes a unique antigen that is completely devoid of the toxic activity of ricin. The thermostabilized vaccine proved 100% protection in preclinical ricin aerosol exposure of mice and primates [39].

Other researches proposed the use of a mixture of 3 proteins to bind and inactivate ricin and the activation of cholinergic anti-inflammatory pathways through the alpha7 nicotinic acetylcholine receptor (nicotine administration of vagal stimulation) in order to reduce proinflammatory genes expression [40].

Ricin as a bioweapon

At the end of the First World War, US initiated a research program with ricin named compound W as a potential replacement of phosgene, the pneumotoxic agent used at that time. When inhaled, ricin proved 40 times more toxic [41].

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. Previously, a military research program conducted in France in 1939 concluded that lungs are not a good pathway of absorption for ricin, but the issue was in fact the thermal effect of the explosion that inactivated ricin. Japan tested ricin on prisoners of war in 1944 [42].

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.

In 1988, Iraqi authorities prepared 10 L of ricin solution to 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 [43].

Portable ricin detectors made for military usage may give false positive results in the presence of other substances. Laboratory testing of samples is needed for confirmation. 0.5% sodium hypoclorite solutions proved efficient for surfaces decontamination [44].

Conclusions

Ricin is an acute threat for population given its possible usage as a bioweapon in terrorist attacks. Castor oil plant is easy to cultivate and harvest and, except US, cultures and processing plants are not supervised. Ricin extraction does not require laborious and costly technique. Ricin is undetectable once in the body (except for urine 3 days after toxin ingestion), poisoning generates nonspecific symptoms and is potentially fatal with no antidote or specific treatment available. Forensic specialists must be aware of symptoms and post-mortem findings in order to make a correct diagnosis of ricin poisoning.

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