General public

Annual newsletter 1

EuroBioTox

YEAR I, N° I FEBRUARY 2018

FOCUS ON... RICIN BIOTOXIN

Ricin...for better and for worse!

Castor oil plant is most known as an ornamental plant, also used for thousands years by humans for its therapeutic properties: difficult delivery, constipation, heartburn. Castor oil was used in ancient Egypt as oil lamp, and during both world wars in the aviation industry. Nowadays, it is used as industrial lubricant, as ingredient in soap, varnish, nylon, skin moisturizer, hair conditioner and so on.

Its beautiful seeds are used as costume jewelries in the Caribbean region. But it was toward the end of WWI that USA thought about its use as a weapon of war. Along with Canada and the UK their work led to the creation of the so called « W bomb ». By the end of WWII, 1700 kg of ricin were produced! But fortunately no biological weapon was used during WWII.

Although about 2000 times less toxic than botulinum toxin, it is considered that that 2 seeds chewed by a child can be fatal.

Only recently antidots were developped (Noy-Porat T. *et al.* (Ref 1), Respaud R. *et al.* (Ref 2)), consisting of anti -ricin IgG.

Ricin could even be used for disease treatment: in 2014, Marszalowicz G.P. *et al.* (Ref 3) reduced mice pulmonary tumor by 80% and improved survival by 25% in mice with established colorectal cancer metastases by injecting a ricin A chain-conjugated monoclonal antibody without histologic evidence of toxicity in normal tissues. Lastly, Pincus S.H. *et al.* (Ref 4) published a paper where an antibody conjugated to ricin-A chain was efficiently used to target HIV infected cells.

Ricin… for better and for worse!

Strategies for ricin detection

Several strategies exist for the analysis of ricin. Techniques such as Enzyme-Linked Immuno-Sorbent Assay (ELISA) or mass spectrometry (MS) can be used for routine analysis in the laboratory, depending on the lab expertise and availability of the required equipment.

ELISA is based on the capture of the toxin (antigen) by a protein (capture antibody) previously fixed on a plate. A second protein (detection antibody), directly or indirectly linked to a signal-emitting enzyme, will recognize the captured toxin. The measured signal is proportional to the toxin concentration. ELISA sensitivity is about 1-10 pg/mL for ricin (Ref 5).

Mass spectrometry is usually used for forensic studies. Ricin is digested by an appropriate protease. Generated peptides are separated on a chromatographic column then ionised and analysed by MS according to their specific mass-to-charge ratio. This specificity permits the identification of ricin, with a limit of detection reaching about 0.6 ng/mL, lower than the corresponding oral LD50 (Ref 6), allowing to prove that a person was orally poisoned with ricin.

New CBRN analytical methods

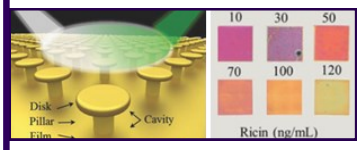


Figure 1, Ref 7

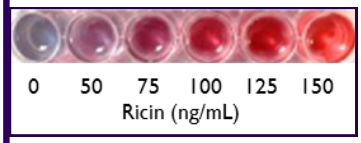


Figure 2, Ref 8

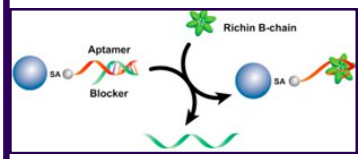


Figure 3, Ref 9

Phage-displayed antibody

Mu X. *et al.* (Ref 7) described a new way to detect ricin with a highly sensitive and specific electrochemiluminescence (ECL)-based immunosensor. The capturing probe consists of gold-magnetic nanoparticles coated with *Staphylococcus* protein A, coupled with anti-ricin polyclonal antibodies. Once ricin gets captured, phage displayed antibodies labeled with the $Ru(bpy)_3^{2+}$ luminophore are added and recognize ricin. The gold magnetic nanoparticles complex is deposited on the surface of a working electrode. A voltage is applied, the ECL reaction between $Ru(bpy)_3^{2+}$-labeled phage displayed antibody and TPA coreactant is triggered, generating photons. The optical signal is detected by a photomultiplier tube detector. This method was successfully applied by the group on ricin spiked complex matrices: river water, biscuits and blood. The linear range of the sensor is $[10^{-7};0.2]$ µg/mL, and the LOD is 0.1 pg/mL.

Metallic colorimetric sensor

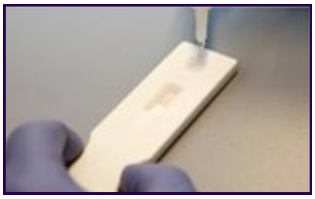
Fan J.R. *et al.* (Ref 8) developed a rapid, *in-situ* colorimetric sensing system based on plasmonic nanopin metasurfaces for ricin quantitative recognition and analysis. After treatment for proper binding, the metallic nanostructure is coated with anti-ricin antibodies and the target ricin molecule is specifically bond to antibody-modified surface after 10 min incubation. The reflection spectra are then characterised with ricin solutions varying from 0.01 to 0.12 µg/mL, generating bright-field reflective colours (figure 1 on the left).

Gold nanoparticles

Sun J. *et al.* (Ref 9) developed magnetic microbeads grafted with copolymer then functionalized with two kinds of capture ligands that permits enrichment of trace amounts of ricin from various complex samples.
After magnetic enrichment and elution, the recovered ricin is readily visually detected with a newly designed gold nanoparticle (AuNP) probe: taking advantage of the depurination activity of ricin, a synthetic substrate consisting of a single-strand 21-mer oligodeoxynucleotide (poly (21dA)) is attached to AuNP. The addition of coralyne, a planar alkaloid that can promote, in absence of ricin, formation of a tight antiparallel homoadenine/coralyne complex, triggers an immediate aggregation of the gold particles, which induced the poly(21dA)-AuNPs solution to exhibit a distinct colour change from red to blue. However, in presence of ricin, the formation of this complex does not happen and the colloid solution remains red (figure 2 on the left).
Assays were performed on drinking water, lettuce, apple juice and human serum spiked with known amounts of ricin. LOD was determined to be 0.038 µg/mL.

Aptamer recognition

Ricin detection using nucleic acid aptamers as recognition element of ricin B-chain was described in two articles, where aptamers immobilised on streptavidin coated magnetic beads are hybridised with a blocker DNA. In presence of ricin B chain, aptamers preferentially link to ricin, making the blocker strand free to pursue the reaction process (figure 3, on the left).
In the first article, Xiao X. *et al.* (Ref 10) developed a proof of concept assay where ricin is detected based on the fluorescence anisotropy (FA) amplification abilities of graphene oxide (GO), and the blocker DNA recycling abilities of Exonuclease III. A fluorescent probe previously attached to the GO is liberated to hybridise with the free blocker DNA. Exo III then digests this fluorescent probe DNA, thus liberating the hybridised blocker that can therefore go through a new cycle. This results in the digestion of a large number of probe DNAs, generating a substantial decrease of the FA value, and achieving blocker assisted enzyme-catalysed signal amplification. Thus, ricin can be detected by the significantly decreased FA. The linear range is [1.0;13.3] µg/mL with a limit of detection of 0.4 µg/mL.
Li C.H. *et al.* (Ref 11) detected ricin based on isothermal polymerase reaction (IPR) where GO is used as low background platform. Blocker DNA hybridises with a dye-modified hairpin probe and triggers the IPR, resulting in high fluorescence intensity. In the absence of ricin, however, the fluorescence of the dye could be quenched strongly by GO, resulting in the extremely low background signal. Thus, ricin B chain could be detected by the significantly increased fluorescence signal. Successfully used for the detection of ricin in human urine, fruit juice and in castor beans, the linear range of this analytical system is [0.75;100] µg/mL and the LOD is 0.6 µg/mL.



LFA for *in-situ* analysis

Lateral Flow Assays (LFA), developed as a first-intention strategy to permit *in-situ* analysis, consist in devices intended to detect a target analyte in samples without the need for specialised and costly equipment. CEA developed such strips for analysis of ricin, botulinum neurotoxins and staphyloccocal enterotoxin B. Other LFAs were also developed by RKI (upper figure, on the left). These tests are based on an immunochemical reaction forming a complex between antigen and specific antibodies. Use of colloidal gold enables visual read-out of the test with an LOD of 0.1 ng/mL. In addition, tests can be measured with a portable reader (lower figure, on the left). This reader allows a semi-quantification of the test-signals. Environmental samples can be quickly analysed and results are obtained within a few minutes.

Ricin at the cell level

"With their work, J Taubenschmid et al. opened the path to a more comprehensive understanding of ricin toxicity at the cellular level."

Ricin path chart

Ricin consists of two polypeptide chains, ricin toxin A chain (RTA) and ricin toxin B chain (RTB). In case of intoxication, RTB binds to the cell surface and ricin is internalized into the Golgi and the Endoplasmic Reticulum (ER). In the ER, the cytotoxic RTA dissociates and translocates across the ER membrane into the cytosol, where it irreversibly inactivates ribosomes. Taubenschmid J. *et al.* (Ref 12) recently confirmed that Fut9 and Slc35c1 genes are responsible for fucosylation of the LewisX carbohydrate in the Golgi apparatus and that ricin, although present in the cytosol, is unable to enter ER and Golgi in cells lacking either enzyme, impairing its toxicity. Their work also confirmed that the lack of fucosylation increases the proportion of sialyl Lewis X within the Golgi by the α3,3-sialyltransferase encoded by St3Gal4 gene, shielding the terminal galactose of the Lewis X carbohydrate. Therefore, ricin enters the Golgi via the displayed galactose residue of the LewisX structure. Impairing its exposition permits a significant decrease in ricin toxicity. Furthermore, their glycoproteomic studies revealed 6 proteins susceptible to both Fut9 and Slc35c1 knock-out, whose disruption by CRISPR/Cas9 permitted an increase of ricin resistance (Ref 13).
With their work, J Taubenschmid and colleagues opened the path to a more comprehensive understanding of ricin toxicity at the cellular level.

Genetically modified ricin

Considering the risks associated with castor bean cropping such as workers exposure or the use of ricin as biothreat agent for bioterrorism purposes, Sousa N.L. *et al.* explored a genetically modified castor bean that would impair the expression of the toxic protein within the seed by the technique of RNA interference (RNAi) (Ref 14). RNAi is a post-transcriptional gene silencing mechanism that regulates the expression of protein-coding genes. Constructs to express self-complementary RNA transcripts form a double strand RNA, which is processed into small interfering RNAs (siRNAs). These siRNAs trigger a sequence-specific mRNA degradation, leading to gene silencing. Transgenic plants were successfully produced, with almost no expression of ricin in the endosperm seeds, compared to about 20 ng ricin/µg of total protein for the non-transgenic plants. This was confirmed by hemagglutination test activity, characteristic of ricin, as well as by cytotoxicity tests and the regular mouse toxicity assay.
However, a number of pieces of evidence support the idea that ricin plays a role in plant defense against pathogens and Insects. Then, the bio-detoxified transgenic castor bean could at first be used to study the ecological role of ricin and RCA120, its homologue, under field conditions.

References

Ref 1: Noy-Porat T *et al*., Toxicon. 2017 Mar 1;127:100-105. Extended therapeutic window for post-exposure treatment of ricin intoxication conferred by the use of high-affinity antibodies. (PMID: 28089771)

Ref 2: Respaud R. *et al*., J Control Release. 2016 Jul 28;234:21-32. Development of a drug delivery system for efficient alveolar delivery of a neutralizing monoclonal antibody to treat pulmonary intoxication to ricin. (PMID: 27173943)

Ref 3: Marszalowicz GP *et al*., Oncotarget. 2014 Oct 15;5(19):9460-71. GUCY2C lysosomotropic endocytosis delivers immunotoxin therapy to metastatic colorectal cancer. (PMID: 25294806)

Ref 4: Pincus SH. *et al*., J Virol. 2017 Jan 18;91(3). Design and In Vivo Characterization of Immunoconjugates Targeting HIV gp160. (PMID: 27795412)

Ref 5: S. Simon *et al*., Toxins (Basel) 2015 Dec; 7(12): 4967–4986. Recommended Immunological Assays to Screen for Ricin-Containing Samples. (PMID: 26703725)

Ref 6: S.R. Kalb *et al*., Toxins (Basel). 2015 Dec; 7(12): 4881–4894. Recommended Mass Spectrometry-Based Strategies to Identify Ricin-Containing Samples. (PMID: 26610568)

Ref 7: Xihui Mu *et al.* Sensors (Basel). 2016 Feb 27;16(3):308. An Electrochemiluminescence Immunosensor Based on Gold-Magnetic Nanoparticles and Phage Displayed Antibodies. (PMCID: 4813883)

Ref 8: Jiao-Rong Fan *et al. Small.* 2017 Jan;13(1). Plasmonic Metasurfaces Based on Nanopin-Cavity Resonator for Quantitative Colorimetric Ricin Sensing. (PMID 27709785)

Ref 9: Jiefang Sun *et al.* Anal Chem. 2017 Nov 3. Fast on-Site Visual Detection of Active Ricin Using a Combination of Highly Efficient Dual-Recognition Affinity Magnetic Enrichment and a Specific Gold Nanoparticle Probe. (PMID: 29058405)

Ref 10 Xiao X. *et al,* Biosens Bioelectron. 2016 Nov 15;85:822-827. Exonuclease III-assisted graphene oxide amplified fluorescence anisotropy strategy for ricin detection. (PMID: 27295569)

Ref 11: Li CH *et al.* Biosens Bioelectron. 2017 May 15;91:149-154. A graphene oxide-based strand displacement amplification platform for ricin detection using aptamer as recognition element. (PMID: 28006682)

Ref12: Taubenschmid J *et al.* Cell Res. 2017 Nov;27(11):1351-1364. A vital sugar code for ricin toxicity. (PMID: 28925387)

Ref13: Stadlmann J *et al*. Nature. 2017 Sep 28;549(7673):538-542. Comparative glycoproteomics of stem cells identifies new players in ricin toxicity. (PMID: 28959962)

Ref 14: Sousa NL *et al*., Sci Rep. 2017 Nov 13;7(1):15385. Bio-detoxification of ricin in castor bean (Ricinus communis L.) seeds. (PMID: 29133924)

Notes