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Evaluation by ELISA of Ricin Concentration in Fluids and Tissues after Exposure to Aerosolised Ricin, and Evaluation of an Immunochromatographic Test for Field Diagnosis

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Abstract

Introduction: We have formerly developed a point-of-care Immunochromatographic Test (ICT) for the diagnosis of ricin pulmonary intoxication, which was intended to be utilised after nasal swabbing. The requested limit of sensitivity for such a diagnosis was calculated but not tested experimentally, and no other sample source was considered. Here, this approach was evaluated on various samples taken from mice intoxicated by a low dose of aerosolised ricin, to push the system to its limits. The best source of sampling for the diagnosis of ricin intoxication was assessed, as was the validity of the ICT.

Methods: Mice were intoxicated by $3LCt_{50}$ of aerosolised ricin and bodily fluids (nasal and lung lavages, blood and urine) were sampled until the 24th hour after intoxication to evaluate their ricin content, using a sensitive ELISA. The ricin concentrations in solid tissues (lungs, liver, heart and kidneys) were also evaluated in ELISA at a late stage, in order to provide a source of samples for forensic diagnosis if needed. In addition, using a standard solution of ricin, the ICT signals were compared to the ELISA. When the ricin concentration in body fluids was above the ICT limit of sensitivity, the rapid test was utilised to assess the best source of sampling and evaluate the validity of this approach.

Results: The ELISA limit of sensitivity was 150ng/L and the ICT provided a semi-quantitative signal when utilised on ricin diluted in Phosphate Buffered Saline (PBS), with a limit of sensitivity close to 1 µg/L. In the animal model, it was observed that the ricin concentration in nasal fluid decreased after intoxication, becoming lower than the ICT sensitivity limit 4 hours after exposure. In contrast, ricin concentration in lung lavage quickly increased until 4 hours and then remained stable until the 16th hour, so that lung lavage was the best sample source during that timeframe. Liver was the best source of solid tissue to sample for diagnosis at the 24th hour. There was also evidence to suggest that urine could be utilised for diagnostic purposes but the ICT was not able to report on this type of sample.

Conclusion: Following inhalation of ricin, nasal wash was a good sample source as formerly calculated, but for a limited timeframe; here, ricin was probably quickly cleared by physiological mechanisms. Lung lavage may be utilised as an alternative body fluid sample source that can be successfully tested by ICT. Liver is the best solid tissue sample source at later stages of poisoning, for forensic diagnosis. More generally, when the pulmonary route of intoxication is suspected, analysis of pulmonary lavage should be considered, even if nasal fluid turns out to be negative. Lung lavage should be made available in field hospitals to provide the trigger to treat ricin pulmonary intoxication, and perhaps also other bio weapons or contaminants by this route.

Keywords: Ricin; Inhalation; Murine model; Distribution; ELISA: Diagnostic; Immunochromatographic tests

Introduction

The threat of natural or voluntary intoxication with various toxins has prompted the development of methods for the detection of these substances. Over the past half century, concern has particularly been expressed about the potential use of the protein synthesis-inhibitory plant toxin, ricin, for malign purposes against military personnel and civilians. An obvious potential route of exposure is by inhalation and this toxin has been shown to produce life-threatening consequences following the delivery of relatively small quantities as an aerosol [1,2]. A consequence of the possible aggressive use of ricin has been the development of medical approaches to protection and treatment; both vaccination and the post-exposure therapeutic use of passive-immunity in the form of antitoxin antibodies have been explored and products are close to being licensed [3].

It would be impracticable to vaccinate the population of a country against the slight chance that some persons might be exposed to ricin, for example in a terrorist attack. Therefore, in many countries, including the UK and France, we would rather favour the application of an antitoxin, on a case by case basis as required. An antitoxin would not be administered as a blanket cover, partly because the injection of quantities of 'foreign' protein may have side effects in a minority of recipients but there are also logistical and economic factors involved. The need for administration of antitoxin to individuals, the so called 'trigger to treat', would ideally be based upon diagnostic evidence of poisoning. A common feature of all instances of poisoning is a therapeutic window, which is the time available within which the application of an antidote, for example, an antitoxin, would be effective. In the case of inhaled ricin, a murine model in our laboratory

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(unpublished observations) indicated that the therapeutic window was between 16 and 24 hours, for complete protection. It is important that in the event of a suspected incident involving the release of aerosolized ricin, appropriate samples from possible victims are screened using a rapid and sensitive diagnostic tool, to provide the trigger to treat with ricin antitoxin. In a previously reported study [4], we demonstrated that an ICT provided a rapid (within 1 hour) and sensitive (to at least 1 μg/L) indication of the presence of ricin prepared from seeds provided by 19 cultivars of *Ricinus communis*.

This type of test would allow the rapid screening of appropriate samples from possible victims of ricin poisoning since it is simple to undertake, has a fast completion time and is very sensitive. In particular, the sensitivity of 1 μg/L, observed formerly for the test evaluated here, is more than a thousand times higher than expected to be required for diagnosis of pulmonary intoxication by ricin in the human.

The purpose of the present study is to establish what fluids and tissue samples would be selected for the detection of ricin after exposure to an aerosol of this toxin, particularly when employing the ICT for a rapid diagnosis. A mouse model intoxicated with $3LCt_{50}$ of aerosolised ricin was chosen and body fluids (nasal and lung lavages, blood and urine) were sampled until the 24th hour. In addition, solid tissues (lungs, liver, heart and kidneys) were also sampled, in order to allow forensic diagnosis if needed. The ricin concentrations in these samples were assessed by a sensitive ELISA, and the ICT was utilised when the ricin concentration was above its sensitivity limit. The capacity of the ICT to diagnose ricin intoxication was established according to the time point and the source of sampling.

Methods

Ricin

For inhalation studies, pure ricin toxin (batch 914/028) was prepared in-house from seeds (*Ricinus communis var Zanzibarensis*) as previously described [1]. *Ricinus* seeds were purchased from Sandeman Seeds S.A.R.L., 7 Route de Burosse, 64350 Lalongue, France.

Animals

Mice used in this study were female Balb/C strain of a bodyweight range of 17-20 g (6-8 weeks old). Animals were habituated to the experimental animal unit for 7 days prior to the use in these studies; all work was undertaken in accordance with the Animal Scientific Procedures Act, 1986. Each group consisted of twelve animals, a total of thirty-six animals for the full study comprising three aerosol exposure groups.

Exposure of mice to aerosols of ricin

Mice were exposed (head only) to an aerosol of ricin toxin at $3LCt_{50}$, in an exposure chamber with a total system air-flow rate of 22.0 L/min. A small-particle aerosol with a mass median aerodynamic diameter of 1.0 μm was generated from a ricin solution of 2000 mg/L in PBS, by passage at a delivery rate of 0.614 mL/min through a Liu-Lee constant output nebulizer [5], at an air pressure of 2.4 Bar. The ricin solution was spiked with sodium fluorescein (20 mg/L) used to estimate aerosol concentration. The aerosol was captured on a glass fibre filter (Whatman PLC, Brentford, Middlesex, UK) during the exposure period of 20 min. Respiratory minute volumes were recorded using a head-out plethysmography system (respiratory monitoring system, EMMS, UK). These data were used to determine the inhaled dose per mouse (μg per mouse) and the approximate retained dose per exposure group was calculated, based on the method reported by Raabe et al. [6]. A detailed description of the apparatus and procedure is given in a review article on inhalation toxicology of ricin preparations at Dstl [3].

Body fluid and tissue and sampling

Groups of mice were culled at various times post ricin inhalation exposure by lethal intraperitoneal injection with sodium pentobarbital (Euthetal). Groups of four mice were culled at 1, 4, 8, 12, 16 and 24 hours and solid tissues examined included lungs (complete with trachea), heart, liver, kidneys, and spleen. Blood samples, nasal washes, lung lavages and urine samples were assessed as fluid samples. Lungs were initially lavaged (see details below) to extract any soluble ricin present in the respiratory tract and respiratory airways. Examinations were not carried out later than 24 hours as this delay was regarded as sufficient for suspicion of ricin intoxication to arise, following clinical signs such as ocular, nasal and pulmonary irritation, or following ricin detection in the atmosphere. Moreover, studies conducted at Dstl indicated that full protection would not be guaranteed if the antitoxin was administered beyond this twenty four hour therapeutic window in this murine model of intoxication [7].

Blood

The thorax was opened and a heparinised blood sample was taken using cardiac puncture immediately after culling. Blood was collected into 1ml anticoagulant blood tubes (TEKLAB, UK, H2230) (lithium heparin, 16 international units per ml) and mixed on a roller for 15 min before centrifugation at 2000 × g for 5 min, to separate plasma and cell fractions. The blood cell pellet was resuspended in 2 ml lysis buffer (Pharm Lyse™ Becton Dickinson).

Nasal wash

Nasal wash was utilised as a substitute for nasal swab, as the latter was regarded as difficult to perform and not equivalent to human nasal swabbing due to the differences in nasal anterior passage size and shape. In order to obtain a nasal wash, on completion of the tissue/organ extractions, the animal's head was removed and a small bulbous ended metal needle inserted in to the top end of the trachea connected to a 1 mL syringe containing 1 mL of sterile PBS + 0.25M galactose. This was used to flush through the nasal passage of the animal and collected into pre-labelled sterile glass receptacle a kept on ice. Sample recovery from the nasal wash was approximately 75-80% of the original wash fluid volume. All samples were maintained on ice following their collection and processed as indicated below, before being stored at -80°C.

Pulmonary lavage

After head removal, the thorax was opened and lungs with trachea were removed, a 3-way valve was used connected to a bulbous ended tube which was placed in the trachea and tied off with surgical thread. Sterile PBS + 0.25M galactose (1 mL) was introduced into the lungs via the trachea, using a syringe in 200 μl volumes. Fluid was gently withdrawn and collected in a second clean syringe via the 3-way valve. Sample volume recovery for lung lavage was 90%. The lung lavage fluid was placed into a sterile glass receptacle and kept on ice. Lung lavage fluid was centrifuged at 2000 x g for 5 min to separate any cells from fluid. The cell pellet was resuspended in 500 μL lysis buffer, aliquotted and stored at -80°C.

Urine

Urine was collected from mice either using metabolic cages (Techniplast UK Ltd) to collect samples during the time course of the

study, or at time of culling if urination occurred then. The observation was divided in four time windows (2 to 4, 4 to 8, 8 to 12 and 12 to 16 hours). Urine collection started at 2 hours post ricin aerosol challenge, after mice were cleaned and returned to their cages, and terminated at 16 hours due to the small urine quantities recovered after that time point. It was decided that urine sample from each animal would be pooled for each time period, because of the relative lack of sample from individuals. The urine collected was placed in o-ringed sealed screw capped micro-centrifuge tubes and stored in a freezer at -80°C until analysis.

Processing of tissues for ricin extraction

Various tissues were removed from each animal as follows: lungs (complete with trachea), heart, liver, kidneys and spleen. All tissues were collected individually and placed in 20 mL sterile universals, which were kept on ice throughout the procedure.

Each organ was rinsed in sterile PBS, blotted on tissue (to remove excess buffer) and weighed. Elution Buffer (EB) was prepared, containing protease inhibitors (Complete® protease inhibitor 11 697 498 001, 1x tablet per 50 ml buffer volume), in PBS containing galactose (0.25M), bovine serum albumin (BSA, 0.1% w/v) and Triton® X-100 (0.1% v/v). The volume of EB added to each sample for homogenisation was calculated using the following formula: [tissue weight (g) ÷ 100] x 150 = volume of EB. The appropriate amount of EB was added to each tissue sample, and homogenisation took place using (IKA Ultra Turrax T10 Basic). After homogenisation, samples were left on ice for 30 minutes before transfer into pre-labelled o-ringed micro centrifuge tubes. Samples were centrifuged (4°C, 10000 x g, 5 mins). Supernatants from each sample were removed, aliquotted and stored at -80°C pending analysis.

Establishing ricin ELISA methodology for biological samples

A sensitive ELISA method, whose limit of detection was 150 ng/L in PBS, was utilized following an initial development to measure ricin in rat tissues following intramuscular dosing [8], then a later revision (using different antibody sets) for the quantification of ricin in rodent tissues following pulmonary and oral dosing [9].

Immunochromatic test strips (ICT)

ICT strips had been prepared at the Centre de Recherche du Service de Santé des Armées (CRSSA) as previously described (Guglielmo-Viret et al. [10]) and were sent to Dstl in cold packaging and then stored at 4°C until usage (within 1 year from preparation). Before use, the strips were marked on the back with appropriate identification information for each sample. Vehicle only controls (phosphate buffered saline containing 0.05% v/v Tween20) were included in each batch of tests. Strips were run as described in a previous publication [4].

The correlation between the previously described ELISA and the ICT strips was tested by reading the absorbance of the strip test and control lines using a densitometer, on a set of ricin standards (100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.19 and 0 µg/L) with replicates of 4. Samples were taken to compare quantities of ricin present by both ELISA and ICT.

The Peak Relative Densities (PRD) of test lines and corresponding control lines for each strip were determined by densitometric interpretation using Quantity One software (Bio-Rad Laboratories, Hemel Hempstead, UK) and compared (testPRD/controlPRD X 100), as described previously.[4] Means and standard deviations of the 4 replicate values were reported for each sample.

Originally, the test was allowed to develop for 20 minutes and then left to dry for at least 20 minutes further, before reading by eye. Pilot studies led to an observation that on leaving the completed strips overnight, some weak or even negative sample had become clearly positive. Therefore, strips were subsequently incubated at 37°C for 30 minutes after running. This drying stage was a development from the published procedure [4].

Results

Ricin purity

Using sodium dodecyl sulphate polyacrylamide gel electrophoresis and staining with coomassie a single band of approximately 65 KDa, ricin toxin was observed to be pure (single band). If a similar electrophoresis gel was stained using silver stain, then, in addition to the main ricin band, faint bands of *Ricinus* agglutinin (approx. 120 KDa) and single chains of ricin toxin (approx. 30 KDa) were also visible (data not shown). The trace presence of agglutinin and breakdown chains were ignored and the concentration of the major component, ricin, in solution (PBS) was assessed by adsorption at 280 nm and interpreted using the molar extinction coefficient of ricin (93900 ± 3300 L/mol/cm); the protein concentration was determined to be 2.2 g/L.

Ricin Inhaled by study mice

All animals survived to the intended cull times (up to 24 h) after dosing. Signs of ricin intoxication were seen from 12 hours onwards; progressing from mild signs at 12 hours to more severe at 24 hours. Mean calculated retained quantities of ricin were 1.60 µg (± 0.12) per mouse, after the inhalation of $3LCt_{50}$. Observations of the calculations for each animal displayed some variation and consequently, 4 mice for each study time point (1, 4, 8, 12, 16, 24 hours) were culled and nasal washes, lung lavages and tissue samples analysed.

Detection of ricin in solid tissues (ELISA)

Of all tested solid tissues, liver accumulated most ricin at 24 hours (196.6 µg/L), lung (21.2 µg/L) accumulated the next highest concentration of ricin (Figure 1). Ricin was also detected in the heart and kidney tissues, at concentrations of between 6.8 and 15 µg/L. Ricin was not detected in the spleen, stomach or small intestine extracts at 24 hours following aerosol challenge.

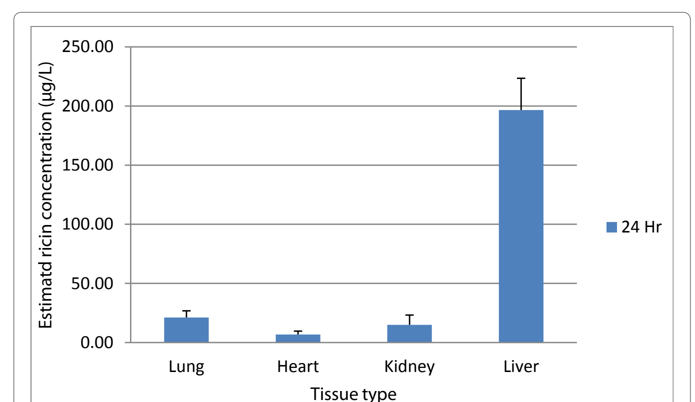

Figure 1: Ricin detected in tissues after murine exposure to $3LCt_{50}$. The figure shows the estimated ricin concentration (µg/L) from tissues where ricin was detected, at 24 hours post ricin aerosol exposure. Bars represent means and standard deviations of 4 replicates. This would indicate that the liver would be the best candidate for post-mortem identification of ricin poisoning.

Detection of ricin in fluids (ELISA)

Ricin was not detected in blood samples or blood cell pellets, but it was detected in urine (Figure 2), with highest concentrations apparently in the 8 to 12 hour window following aerosol exposure.

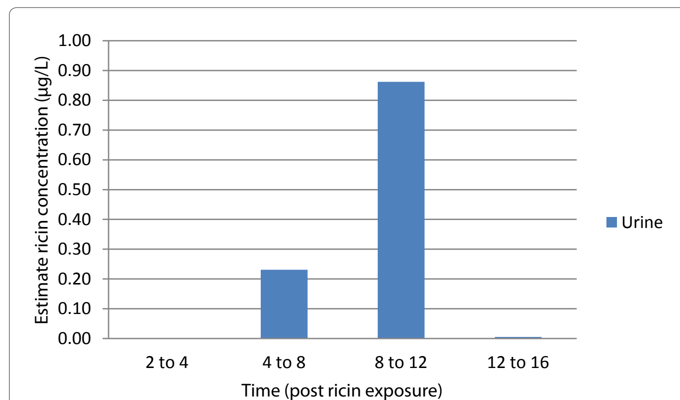

Figure 2: Ricin detected in urine after murine exposure to $3LCt_{50}$. Ricin concentrations detected by ELISA in pooled urine samples collected over four time periods post exposure, from Balb/C mice following a $3LCt_{50}$ inhalation ricin aerosol challenge by ELISA.

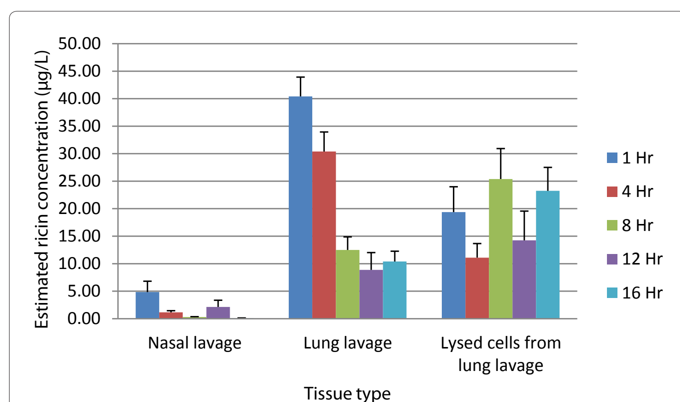

Figure 3: Ricin detected in fluids after murine exposure to $3LCt_{50}$. The figure shows the estimated ricin concentration (µg/L) by ELISA from nasal lavage, lung lavage and lysed cell pellet recovered from lung lavage (sampled at various times post ricin aerosol exposure. Bars represent means and standard deviations of 4 replicates.

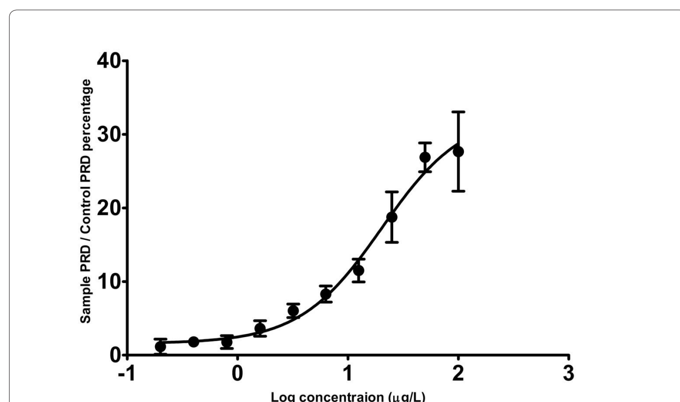

Figure 4: Correlation between ELISA and ICT signals read by a densitometer. The figure shows the ICT Sample Peak Relative Density readings / Control Peak Relative Density plotted as a 4 parameter logistic curve.

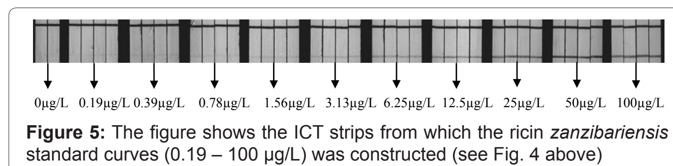

Figure 5: The figure shows the ICT strips from which the ricin *zanzibariensis* standard curves (0.19 – 100 µg/L) was constructed (see Fig. 4 above)

One hour following aerosol exposure, ricin was detected in nasal lavage fluids (4.8 µg/L), in the lung lavage fluid (40 µg/L) and in lysed cells (19.4 µg/L) (Figure 3). At 4 hours after exposure, concentrations diminished by 75% in nasal wash (from 4.8 to 1.1 µg/L) but decreased by 25% in lung lavage fluid (from 40 to 30 µg/L) and in the lung cell pellet (19.4 to 11.1 µg/L). At the 16th hour time point the ricin concentration in lung wash and lung cell pellets were 10 and 23 µg/L while nasal wash levels were below the minimum detectable limit of the ELISA.

Comparison of ICT and ELISA results

A standard curve was produced which is shown transformed to a four parameter logistic curve (Figure 4), using densitometric data (Table 1), obtained from ICT test strips (Figure 5) using ricin standard solutions. This curve was used to extrapolate densitometric data for ICT run with samples extracted from animal tissues after exposure to ricin. At a low ricin concentration (below 3.4 µg/L ricin by ELISA), the variability of the SD for ELISA data was 10.85% of the mean while that for the ICT was 31.7%. At higher ricin concentrations (from 6 to 16 µg/L), variability of the ELISA remained constant (10.0% of mean SD) but increased (42.7% of mean SD) for ICT.

All ICT with peak relative density ratios, which were around 1 or above, were visible to the naked eye. ICT's performed on nasal washes at 1 hour after exposure, were positive for ricin and in some animal samples at 4 hours (data not shown), but all others were negative (Table 2). All ICT performed on lung washes were positive until the 16th hour, showing a marked decrease between the first sample taken immediately after exposure and the second one, taken 8 hours later, then decreased to become negative at the 24th hour (Table 2).

Discussion

This study was divided in two parts: The first part used ELISA to measure ricin concentrations found in fluids and in tissues after inhalation of this toxin, and the second part evaluated the feasibility of an ICT for ricin to diagnose exposure. This first part helped design the second part, as the ICT was compared with the ELISA to assess its sensitivity limit on a standard ricin solutions in PBS, and the ICT was then used to test for ricin in bodily fluids.

In the first part of the study, liver and lung were shown to be the best tissue sources for the detection of ricin after inhalation. No ricin was detected in the blood by ELISA or ICT. The fact that ricin was quantified in liver and kidneys (and also in the heart) indicates that ricin does cross the alveoli to enter the blood stream but at levels below the detection limits of our assays. A previous study [11] examined the localisation of ricin following inhalation nose-only challenge of mice with ^{125}I-labelled ricin delivered using a Collison nebuliser, similar to our study. Radiolabel was detected in many tissues and the authors determined 'Ricin Equivalent' (RE) concentrations from the radioactive counts measured. However, the authors discussed the likelihood that the radioactivity measured in tissues could well have been free ^{125}I rather than labelled ricin. When ricin equivalents per milligram were measured in blood the maximum concentration detected was about 5.4 pg/mg, which is below the limit of detection of our ELISA and ICT. This might explain why we could not detect ricin in the blood.

0 μg/L	0.19 μg/L	0.39 μg/L	0.78 μg/L	1.56 μg/L	3.13 μg/L	6.25 μg/L	12.5 μg/L	25 μg/L	50 μg/L	100 μg/L
0	1.777 ± 1.043	1.793 ± 0.344	1.756 ± 0.860	3.632 ± 1.044	6.040 ± 0.890	8.294 ± 1.120	11.525 ± 1.558	18.749 ± 3.389	26.854 ± 1.957	27.639 ± 5.401

Table 1: The Peak Relative Density values with the background subtracted sample band or buffer control ICT strips are shown. Data represented by the mean ± standard deviation used to construct the standard curve (Figure 4).

Chip ID	Time Point (Hrs)	ELISA data	Mean	SD	ICT data	Mean	SD
8296	1		35.211	3.510		31.590	6.089
5489	1		25.560	3.558		21.818	9.537
7103	8		6.603	0.293		4.194	2.559
8940	8		13.311	1.192		29.601	2.171
9433	8		16.043	4.313		15.319	2.047
7437	8		12.991	0.943		8.053	2.431
1264	12		16.813	0.293		18.643	7.281
1852	12		3.402	0.269		4.231	2.768
8733	12		14.806	0.786		19.577	6.670
1142	12		0.420	0.020		ND	ND
1744	16		7.335	0.558		7.398	4.238
5325	16		10.278	1.457		19.068	7.402
8313	16		10.810	1.758		17.412	11.373
8951	16		12.831	0.985		13.767	3.669
4678	24		0.552	0.296		NA	NA
9769	24		0.212	0.076		ND	ND

Note: ND sample not detected by ICT. NA samples were not analysed by ICT

Table 2: Ricin detected in lung lavage at various time points after exposure to $3LCt_{50}$. The table shows the levels of ricin detected in individual mice analysed both by ELISA and ICT (μg/L), 1, 8, 12, 16 and 24 hours after exposure.

Regarding nasal fluid, the rapid decrease of ricin concentration in nasal wash came as a surprise, and it may be due to mucociliary clearance [12]. We did not take into account this clearance when we calculated the sensitivity limit of the ICT, and it underlines that calculated values in biology should be verified by in-vivo experiments. Ricin concentrations in lung lavages were around 10 μg/L or higher and this sample source appears to be suitable for testing by ICT from between 4 and 16 hours post exposure. The concentration of ricin in lung lavage decreased with time, possibly due to mucociliary clearance and the ingress of ricin in to solid lung tissue; pulmonary oedema or leakage of ricin into the circulation may also be an explanation for ricin loss from the lungs at later stages.

It was suggested by Guglielmo-Viret et al. [10] that nasal swabs could provide useful sample sources for the detection of ricin following inhalation, using ICT strips. An ICT was specially developed for this purpose and, in the present study, it was positively evaluated as a semi-quantitative test and its sensitivity was confirmed. The ICT was evaluated in-vivo in the second part of the present study, using a murine model of intoxication by aerosolized ricin. In this model, nasal swabbing cannot easily be performed and it was replaced by a nasal wash, which is likely to be more efficient. The use of fairly low challenge dose of ricin, $3LCt_{50}$, pushed the model to its limit. After inhalation ricin concentrations in nasal wash were low, as estimated by ELISA. In particular, only nasal washes performed one hour after aerosol challenge gave positive ICT results. At all time points however, the ICT gave positive results when utilized on lung lavage samples. These results might be evaluated in a larger animal model, where swabbing of nostrils would be a possibility. However, results tend to indicate that, in a situation where ricin intoxication is suspected by clinical signs or atmospheric surveillance, lung lavage should sampled and tested by ICT, even if the nasal swab tests turn out to be negative.

The present study evaluated the use of another biological fluid, urine, for ricin detection. Urine pooled from 8 to 12 mice at between 2 and 16 hr time points was tested by the ELISA, which possibly detects degraded ricin; the ricin levels determined were approximately 0.22 (4 to 8 hr period), 0.86 μg/L (8 to 12 hr period) respectively. These concentrations are below or near the limit of ricin detection by the ICT. Applying the same reasoning as for nasal washes, ricin detection by ICT in urine should also be tested in a larger animal model.

The closest model for humans is assumed to be the non-human primate, where ricin LD_{50} might be equivalent [13] or three-fold higher [3] than in mice (3-5 μg/kg). It might thus be assumed that for humans, the lethal inhaled dose of ricin is equivalent or higher than for mice, but require quantitatively more ricin on a dose/kg bodyweight basis. Based on their relative sizes, the lethal dose for a human of 70 kg would be 3,500 times greater than for a mouse of 20 gr. If the ricin concentration in nasal wash is in proportion with this ratio, this would mean that levels associated with human inhalation poisoning should be well within detectable range of IC`T assay in nasal washes but the timeframe for this detection has to be established. The possibility of detecting ricin with ICT on nasal washes should thus be experimentally tested on a larger species than the mouse, preferably a non-human primate model.

Conclusions

Using a mouse model of pulmonary intoxication by low doses of ricin, it was shown that nasal swabbing might only give positive results at an early time, and that lung lavage may be preferred between 4 and 16 hours post exposure. By 24 hours, ricin concentration in lung lavage

decreased by about 75% but at such a late time, forensic diagnosis may have to be considered; it was shown here that liver might be the best source of samples for such procedure. The present results should be evaluated in a larger animal model, preferably a non-human primate. These first results indicate that when nasal swabs give negative results, lung lavage may be recommended and perhaps made available in military field hospitals for triage purposes.

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