Forensic Strategy for the Detection of Plant Toxin Ricin

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ABSTRACT

The forensic strategy for determination of ricin is proposed. On-site detection is performed for on-site evidence samples using lateral flow immunoassay kit. Laboratory preliminary analysis is performed for the transported evidence samples to detect ricin as the standpoint of molecular weight criteria, using sodium dodecyl sulfate polyacrylamide gel electrophoresis, μchip gel electrophoresis or matrix-assisted laser desorption ionization-time of flight-mass spectrometry. Final identification is performed using liquid chromatography-electrospray ionization tandem mass spectrometry for tryptic peptides of ricin sample.

Key words: ricin, lateral flow immunoassay, gel electrophoresis, MALDI-TOF-MS, LC-MS, tryptic digestion.

INTRODUCTION

Considering the recent situations involving chemical [1] and biological [2] warfare terrorism, the threat of terrorism using proteinous toxins is now emerging, and so, it is necessary to establish the analytical methods for determining proteinous toxins, such as botulinum toxins, Staphylococcal enterotoxin B and ricin [3]. However, the methodology for determining high-molecular weight compounds has not been established in forensic toxicological field. Ricin, which is produced in caster bean, is a heterodimer where ribotoxin is linked with a galactose-binding lectin via a disulfide bond [4]. Our laboratory has established both the on-site detection [5, 6] and laboratory analysis system [7-9] for ricin in the standpoint of homeland security and forensics. Here, we propose the forensic strategy for detection and identification of ricin using immunochemical, biochemical and mass spectrometric technologies.

EXPERIMENTAL

Ricin was provided from the Honen Corporation, and used under the permission of the Minister of Economy, Trade and Industry. The Bio Threat Alert^{TM} (BTA) Test Strips for "Ricin" were obtained from Tetracore LLC. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed using 10% gel and 2-mercaptoethanol reduction [10]. Microchip (μchip) gel electrophoresis (μChip GE) was performed by 2100 BioAnalyzer (Agilent Technologies) using the Protein 200 Plus Assay kit. Ricin was denatured by heating with SDS in the presence or absence of reducing reagent, and applied to the above gel electrophoretic analysis. Matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) was performed using Voyager-DE^{TM} STR (Applied Biosystems) with linear mode and positive polarity using 5 mg/ml sinapinic acid in acetonitrile-water (1:3 by v/v) as matrix. Ricin was denatured with 7 M guanidine-HCl,

reduced with 0.25% dithiothreithol, alkylated with 0.6% iodoacetic acid for 2.5 hr at 25°C, digested with 5 μg/ml trypsin for 24 hr at 37°C, and analyzed by liquid chromatography-mass spectrometry (LC-MS) using 1100 LC system equipped with MDS Trap (Agilent Technologies) with the following instrumental conditions (column: Zorbax SB C18 300A (0.5 mm x 150 cm, 5 μm), acetonitrile-aqueous trifluoroacetic acid (TFA, 0.05%) gradient elution, 10 μl/min, electrospray ionization (ESI, positive)).

RESULTS AND DISCUSSION

Ricin was detected by the BTA system with the limit of detection (LOD) of approximately 0.1 μg/ml, and white powder compounds such as wheat flour did not interfere with the detection. The procedure was easy and it took only 15 min throughout the procedure. Therefore, this commercially available lateral flow immunoassay (LFI) kits can be used as field method for detecting ricin.

From authentic ricin, two protein bands (60 kDa) were observed by non-reduced SDS-PAGE and μChip GE, and from reduced authentic ricin, several protein bands (30 kDa) were observed. The LOD were 240 and 9 ng/ml, for PAGE and μChip GE, respectively. *Ricinus communis* agglutinin, which molecular structure is similar to ricin and contained in caster bean, showed different behavior in the gel electrophoresis (60 and >100 kDa bands in non-reduced; three bands around 35 kDa in reduced conditions). Comparing with the electrophoretic mobility of the molecular weight makers, it was possible to detect ricin without authentic sample. However, because ricin is the main ingredient in crude caster bean extract, it is possible to use the caster bean extract as the standard. It took several hours throughout the analysis.

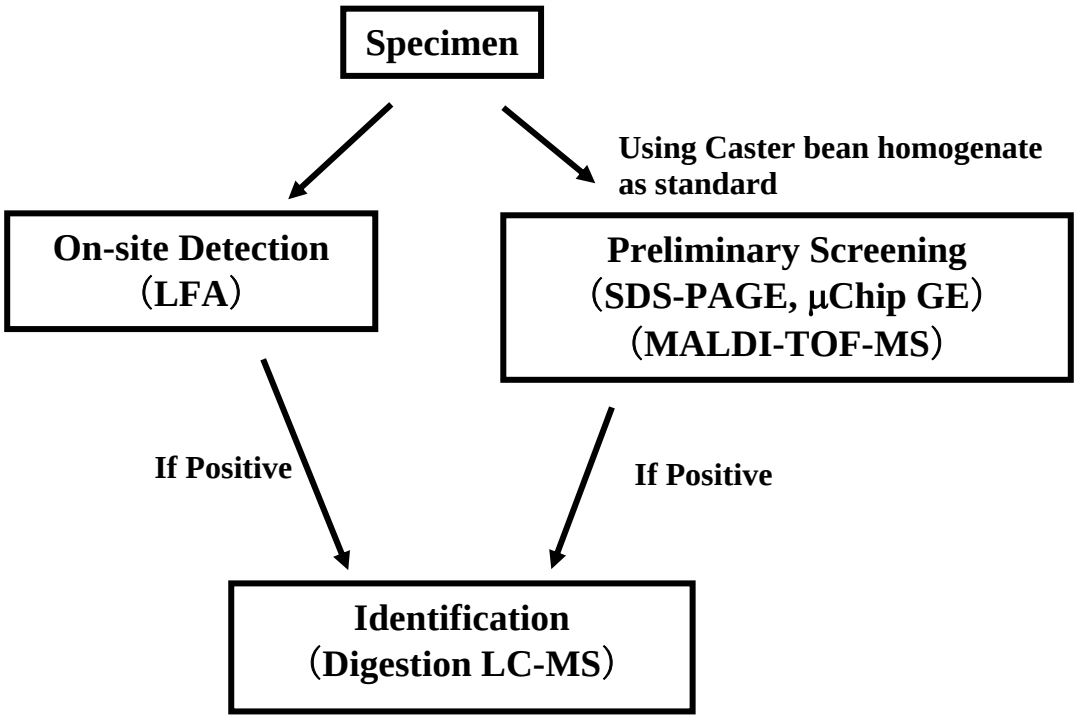

Fig. 1: The proposed forensic toxicological strategy for determining ricin.

Two distinct peaks, singly (*m/z* 62,592) and doubly (*m/z* 31,401) protonated molecular ions were detected on the mass spectrum of authentic ricin by MALDI-TOF-MS, and two partially-overlapped peaks around *m/z* 31,000-32,000 were observed for reduced authentic ricin. The procedure was simple and it took only several minutes throughout the analysis.
LC-MS (capillary ODS column, acetonitrile gradient elution, ESI) analysis for intact ricin showed broad and unclear mass spectrum. Instead, the trypsin-digested ricin gave multiple peptide peaks on LC, and the distinct ESI mass spectra were obtained from 13 peaks, all giving singly and doubly protonated molecular ions. From the MS/MS analysis, clear peptidic fragmentation patterns were observed, leading to peptide sequencing. The LOD was obtained to be 6 ng per injection. This identification result is compatible with the paper of another forensic science group [11]. The digestion LC-MS can be used as identification method for proteinous toxin ricin.
Fig. 1 shows the proposed flow-chart of forensic toxicological technologies for the determination of ricin. LFI can be performed by first responders for on-site evidence samples such as suspected white powders. If positive results are obtained, such samples can be sent to forensic science laboratories, and examined by specialists using digestion LC-MS. Instead, on-site samples can be directly sent to forensic science laboratories, and screening tests can be
performed using SDS-PAGE, μChip GE or MALDI-TOF-MS. If positive results are obtained, such samples can be examined using digestion LC-MS.

References

1. Y. Seto, N. Tsunoda, M. Kataoka, K. Tsuge, T. Nagano, Toxicological analysis of victims' blood and crime scene evidence samples in the sarin gas attack caused by the Aum Shinrikyo Cult. in: A.T. Tu, W. Gaffield (Eds.), Natural and Selected Synthetic Toxins - Biological Implications, American Chemical Society, Washington, DC, 1999, p. 318-332.
2. T. V. Inglesby, T. O'Toole, D. A. Henderson, J. G. Bartlett, M. S. Ascher, E. Eitzen, A. M. Friedlander, J. Gerberding, J. Hauer, J. Hughes, J. McDade, M. T. Osterholm, G. Parker, T. M. Perl, P. K. Russell, and K. Tonat, Anthrax as a biological weapon, 2002.J. Amer. Med. Assoc. 287 (2002) 2236-2252.
3. Center for Disease Control and Prevention, Categorization of bioterrorism agents of public health importance, www.bt.cdc.gov/agent/agentlist-category.asp (2000).
4. J. M. Lord, L. M. Roberts, J. D. Robertus, Ricin: structure, mode of action, and somecurrent applications. FASEB J. 8 (1994) 201-208.
5. K. Iura, K. Tsuge, Y. Seto, A. Sato, Detection of proteinous toxins using the Bio Threat Alert system. Jpn. J. Forensic Toxicol. 22 (2004) 13-16.
6. K. Tsuge, I. Ohsawa, K. Matsushita, H. Sekiguchi, Y. Seto, A. Sato, Detection of proteinous toxins using the Bio Threat Alert system, part II. Responses to high levels of toxins and interferences by colored sample matrices. Jpn. J. Forensic Toxicol. 23 (2005) 18-20.
7. M. Kanamori-Kataoka, Y. Seto, Simultaneous and rapid analysis of nerve gases and proteinous toxins by liquid chromatography/mass spectrometry. Jpn. J. Forensic Toxicol. 23 (2005) 21-28.
8. Y. Seto, M. Kanamoti-Kataoka, Mass spectrometric strategy for the determination of natural and synthetic organic toxins. J. Health Sci. 51 (2005) 519-525.
9. M. Kanamori-Kataoka, I. Ohsawa, Y. Seto, Analytical method for proteinous toxin ricin based on its molecular weight estimation. Jpn. J. Forensic Sci. Technol. 11 (2006) 131-138.

10. U. K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227 81970) 680-685.
11. S. A. Fredriksson, A. G. Hulst, D. Artursson, A. L. de Jong, C. Nilsson, B. L. van Baar, Forensic identification of neat ricin and of ricin from crude caster bean extracts by mass spectrometry. Anal. Chem. 77 (2005) 1545-1555.