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Detection and measurement of carbohydrate deficient transferrin in serum using immuno-capture mass spectrometry: Diagnostic applications for annual ryegrass toxicity and corynetoxin exposure

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ABSTRACT

The neurological livestock disease annual ryegrass toxicity (ARGT) is caused by the ingestion of the naturally occurring glycolipid toxins – the corynetoxins. Corynetoxins also threaten human health as potential contaminants of the food supply. Presently, there are no routine diagnostic tests for corynetoxins-exposure in humans or livestock. Chronic ingestion of corynetoxins has been modeled in rats exposed to dietary tunicamycins for 12 months and carbohydrate deficient transferrin (CDT) has been previously identified as a candidate disease biomarker. Here, the technique of immuno-capture mass spectrometry (icMS) was used to evaluate serum levels of CDT, discriminating between control and tunicamycins-exposed rats with 85% accuracy. The icMS approach is based on the combination of specific transferrin enrichment with functionalized magnetic beads and automated matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). With no other clinically-relevant diagnostic tests available icMS could be readily adapted for high-throughput clinical assessment of corynetoxins-exposure in humans or livestock.

1. Introduction

Annual ryegrass toxicity (ARGT) is an often-fatal neurological disease of significant importance to Australia's valuable agricultural sector. ARGT in grazing livestock is caused by the ingestion of corynetoxins (CT) in contaminated pasture (Finnie, 2006). The causative agents of ARGT have also been identified in seed gall material from New Zealand and the USA (Anderton et al., 2004). A syndrome that is clinically and pathologically indistinguishable from ARGT can be manifested experimentally using the commercially available tunicamycins (TM) since both CT and TM inhibit oligosaccharide biosynthesis in the protein *N*-glycosylation pathway in a toxicologically equivalent manner (Jago et al., 1983). As well as impacting upon animal health CT also threaten humans as potential contaminants of the food supply via tainted grains or food products derived from subclinically-exposed animals. While there have been no recorded cases of human illness directly attributed to CT the effects of low-level exposure are likely to be latent, chronic and difficult to distinguish from common health problems such as infertility, miscarriage, immunodeficiency and neuropathology (Edgar, 2004). This necessitates the development of a high-throughput, non-invasive diagnostic test for detecting CT exposure that is compatible with both humans and livestock. Candidate serum biomarkers of long-term, low-level exposure to TM, a model for chronic CT poisoning, have already been identified using two-dimensional electrophoresis (2D-E) (Penno et al., 2009a). Transferrin emerged as a particularly promising marker based on a population of carbohydrate deficient transferrin (CDT) that was detected in the toxin-exposed but not the control rats.

The quantification of protein and peptide biomarkers has traditionally involved Western blotting or ELISA (Sparbier et al., 2009). Western blotting can be used to differentiate between specific protein isoforms based on molecular weight and/or isoelectric point shifts in the research laboratory but the process lacks the throughput required in a clinical setting. Conversely, the diagnostic ELISA is often unable to discriminate between post-translationally modified proteins and peptides that may be clinically relevant. Over the past decade, profiling for disease-specific protein/peptide signatures in biological fluids by mass spectrometry (MS) has become a popular concept for disease diagnosis. A traditional MS profiling platform consists of a chromatographic separation strategy for protein/peptide enrichment (e.g., surface-enhanced laser desorption/ionization [SELDI] chips or functionalized magnetic beads), a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF MS) mass spectrometer for analyte detection, and specialized software for

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data interpretation. The application of MS profiling as a tool for biomarker discovery remains controversial with several hundred papers either promoting or refuting its diagnostic potential. While the technique has the advantages of small sample volumes, high-throughput capacity, compatibility with automation and an optimal working range for low molecular weight proteins and peptides, two major criticisms of MS profiling are its sensitivity to non-biological variation and potential lack of specificity. Poor specificity has been associated with solid-phase capture devices based on non-specific surface chemistries (e.g., immobilized metal affinity chromatography, hydrophobic interaction chromatography, anionic/cationic exchange etc.) capturing only the most abundant proteins/peptides present in μg–mg/mL concentrations in a sample. Accordingly, proposed signature peaks associated with particular physiological states have been subsequently identified as non-specific, acute phase reactants rather than specific peptides and/or proteins released by tumors or diseased tissues, thus reflecting the consequence of a diseased state rather than the disease specifically (Diamandis, 2006), or may represent markers of sample handling variability (West-Norager et al., 2007).

Immuno-capture mass spectrometry (icMS) incorporates specific antibodies into the chromatographic separation step for targeting and enriching known biomarkers in clinical samples. The high resolution and mass accuracy of modern MALDI-TOF mass spectrometers, which are already in clinical usage for microbial identification (Seng et al., 2010), then enables the discrimination of targeted protein/peptide isoforms in a high-throughput manner. Consequently, icMS retains the many advantages of MS profiling while improving specificity and sensitivity. In this present report, an icMS-based assay for detecting and measuring CDT in serum, and hence providing a potential diagnostic tool for detecting CT exposure, is proposed.

2. Materials and methods

2.1. Animals

Experimental procedures involving animals were performed in compliance with approved protocols and ethical standards set by the Australian Animal Health Laboratory's Animal Ethics Committee (Geelong, Australia. AEC Protocol 879). Ten rats (five male, five female) were exposed to 40.5 μg/kg body weight/day of TM in pelleted food for 12 months as previously described (Penno et al., 2009a). A control group also consisting of 10 rats (five male, five female) was given equivalent food without the toxin. Serum samples were collected and diluted 1/7 in ultrapure water (18.2 MΩ-cm) to contain ~10 μg/μL of protein as previously described (Penno et al., 2009a), frozen rapidly in small aliquots and stored at constant −80 °C (monitored freezer) for five years until required for this investigation.

2.2. ELISA

ELISA protocol was based on a previous method (Kottgen et al., 1988). Wells of a Nunc-Immuno MaxiSorp 96-well plate (Nunc, Roskilde, Denmark) were coated with a 1/80,000 dilution of rat serum and probed with a 1/10,000 dilution of rabbit anti-rat transferrin (Tfn) antibody (henceforth anti-Tfn IgG; lyophilized IgG fraction reconstituted to contain 14.5 mg/mL total protein as stipulated by manufacturer in 0.05% thiomerosal, 1% glycerol; catalogue number 55734; MP Biomedicals, Irvine, CA, USA) followed by a 1/20,000 dilution of goat-anti-rabbit IgG-HRP conjugate (Calbiochem, San Diego, CA, USA). The color reaction was developed with K-Blue TMB substrate (Elisa Systems, Brisbane, Queensland, Australia) and stopped with 50 μL/well of 0.5 M sulfuric acid. The plate was scanned at 450 nm using a Titertek Multiskan Plus MKII microplate spectrophotometer (Labsystems, Helsinki, Finland). Each sample was measured in duplicate and averaged.

2.3. Magnetic bead fractionation

For all 20 rat serum samples the magnetic bead extraction process was performed in triplicate. ClinProt MB-IAC Prot G magnetic beads were processed as described by the manufacturer (Bruker Daltonics, Bremen, Germany) using modified volumes of the required solutions. Washing, immobilization, binding and elution buffers were supplied with the MB-IAC Prot G antibody capture kit. Briefly, in Step I, the reconstituted anti-Tfn IgG (3 μL undiluted) was coupled to the beads (7.5 μL of suspended bead solution) in immobilization buffer (22 μL). After washing using the magnetic separation device (Bruker Daltonics) the beads were resuspended in binding buffer (10 μL) together with 5 μL of diluted rat serum (refer to Section 2.1) containing approximately 50 μg of protein (Step II). Following incubation for two hours at room temperature and thorough washing, elution buffer (5 μL) was applied to the beads and the supernatant removed (Step III). A 2.5 μL aliquot of this supernatant was mixed with 2.5 μL of sinapinic acid (10 mg/mL in 90% acetonitrile, 0.1% trifluoroacetic acid in water) and spotted on three positions (1 μL each) of an 800 μm AnchorChip target (Bruker Daltonics) for subsequent MALDI-TOF MS analysis. The remaining material was stored at −80 °C.

2.4. MALDI-TOF MS

As stated in Section 2.3, sera from each rat was subjected to the magnetic bead extraction process in triplicate and each magnetic bead eluate was analyzed in triplicate resulting in nine MALDI spectra per sample (Callesen et al., 2008). Accordingly, 180 mass spectra were collect in total; 90 for control and 90 for TM-exposed rats. Spectra were automatically acquired using an UltraFlex III MALDI TOF/TOF (Bruker Daltonics) operating in positive linear ion mode between *m/z* 10,000 and 160,000 under the control of Bruker Daltonics' FlexControl (v 3.0) and AutoXecute (v 3.0.100.0) software packages. Instrument and software-specific settings are described in Supporting Information Table T1. The mass spectrometer was calibrated externally every fourth sample using Bruker Daltonics' Protein Standard II. Data analysis was performed with FlexAnalysis (v 3.0, Bruker Daltonics) and ClinProTools (v 2.1, Bruker Daltonics) using settings outlined in Supporting Information Table S1.

2.5. Tryptic digestion

A single bead eluate (1 μL) from a TM-exposed rat was transferred to a fresh microfuge tube, left to evaporate at room temperature (<1 h), and sequencing grade modified trypsin (Promega, Madison, WI, USA; 3 μL of 1 ng/μL in 10 mM NH_4HCO_3/20% acetonitrile) was added. The sample was incubated at 37 °C for one hour then the entire volume was spotted onto a 600 μm AnchorChip (Bruker Daltonics). A matrix of α-cyano-4-hydroxycinnamic acid (CHCA; Bruker Daltonics) was applied (Zhang et al., 2007) and MALDI-TOF MS and MS/MS spectra in reflectron mode were acquired using the UltraFlex III as described previously (Penno et al., 2009a). Mascot database searching was performed using the following parameters: taxonomy = Mammalia (mammals) (64438 sequences), database = SwissProt 57.1 (462764 sequences; 163773385 residues), enzyme = trypsin (maximum two missed cleavages), variable modifications = oxidation of methionine, peptide mass tolerance = ±100 ppm, fragment mass tolerance = ±0.8 Da.

2.6. PNGase F digestion

PNGase F (New England Biolabs, Ipswich, MA, USA; 12 U in 0.24 μL of storage buffer [50 mM NaCl, 20 mM Tris–HCl pH 7.5, 5 mM Na_2EDTA, 50% glycerol]) was added to 6 μL of diluted control rat serum and incubated overnight at 37 °C. An untreated sample from the same animal was prepared with the addition of storage buffer (0.24 μL) without PNGase F. SDS–PAGE and CBB staining were performed as described previously (Penno et al., 2009a).

3. Results

3.1. ELISA

The OD_{450nm} readings of the total serum transferrin ELISA for the 10 control and 10 TM-exposed rats are graphically summarized in Fig. 1A. The mean value of the TM-exposed group was not significantly different from the controls as determined by a Student's *t*-test ($p = 0.06$).

3.2. Evaluating spectrum validity

To evaluate whether each acquired mass spectrum was a true representative of the sample or, for example, contained abnormally high or low peak masses for either the transferrin or CDT peaks, all 180 mass spectra were processed using FlexAnalysis with settings outlined in Supporting Information Table S1. Based on automatic peak detection, the doubly charged anti-Tfn IgG and singly charged transferrin peaks (as highlighted in the average spectra presented in Fig. 1B) were identified in 67/90 (74%) and 88/90 (98%) of mass spectra from the control rats and 75/90 (83%) and 90/90 (100%) of mass spectra from the TM-exposed rats, respectively. The singly charged CDT peak was automatically detected using FlexAnalysis in 35/90 (39%) of the TM-exposed mass spectra, but, as expected, in none of the 90 control mass spectra. Where peaks were not automatically detected in a mass spectrum they were identified manually, including in the appropriate *m/z* region corresponding to the CDT peak for both control and TM-exposed spectra, for comparative statistical purposes. Peak lists and parameters were exported to Office Excel 2003 (Microsoft) and the intensities of the transferrin and CDT peaks were normalized by dividing their values by the intensity of doubly charged anti-Tfn IgG peak for that mass spectrum, which served as an internal standard. A Dixon's *Q*-test based on a 95% confidence interval was performed on normalized transferrin and separately on the normalized CDT values of the nine replicate spectra for each biological sample. Three mass spectra (two from control rats and one from a TM-exposed rat) were identified as outliers based on the transferrin peak and one spectrum from a TM-exposed rat was an outlier based on the CTD peak. These outlier spectra were removed and not used in the production of average mass spectra (described in Section 3.3), comparative statistics (Section 3.4), model generation (Section 3.4) or subsequent classification in ClinProTools (Section 3.4).

3.3. Average mass spectra

Average control and TM-exposed mass spectra generated in ClinProTools based on all valid replicate spectra are shown in Fig. 1B. The major peaks in the spectra were assigned to singly and doubly charged transferrin (*m/z* 77,000 and 39,000,

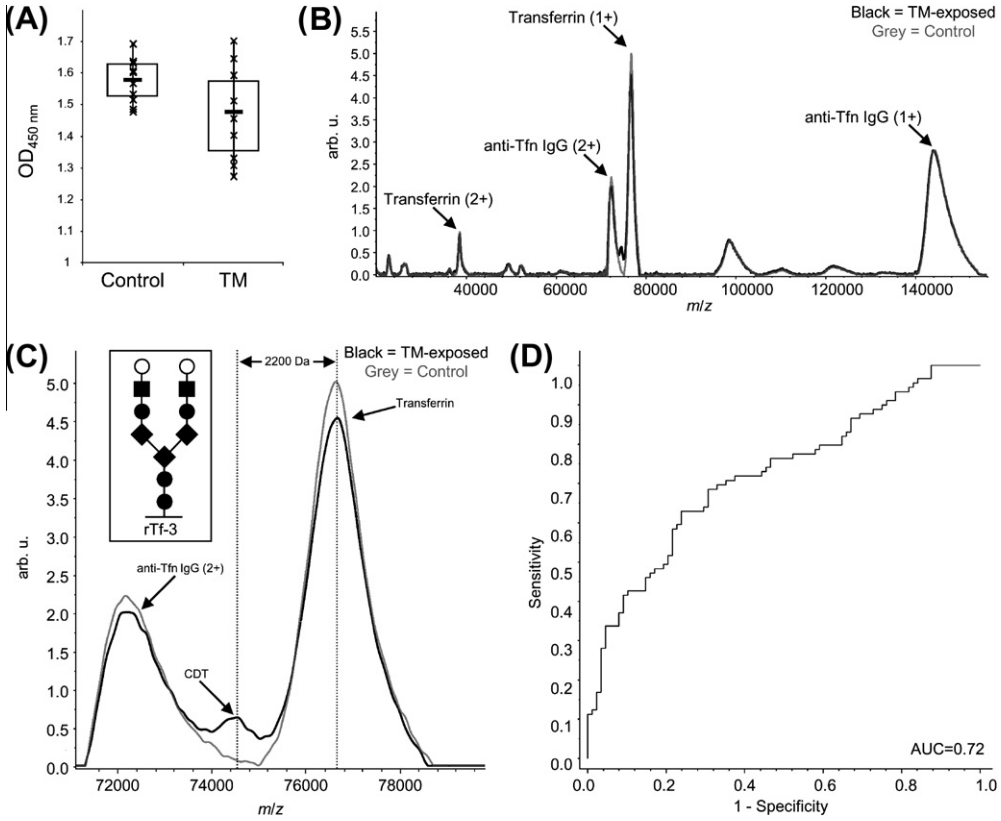

Fig. 1. (A) Results of the total serum transferrin ELISA. (B) Average mass spectra produced in ClinProTools for control and TM-treated rat sera. Each is based on 90 spectra whereby nine replicate mass spectra were obtained for each of the 10 biological replicates per group. (C) Enlarged view of the spectrum highlighting the mass difference between the singly charged transferrin and CDT peak corresponding to the rTf-3 carbohydrate side chain (insert). ● = GlcNAc, ▲ = Man, ■ = Gal, ○ = Neu5Ac(α2–6). (D) ROC curve produced in ClinProTools for the CDT peak.

Table 1
Determining the intra- and inter-replicate CV% values of the normalized transferrin peak intensity in mass spectra of control and TM exposed rats.

Group	Rat ID	Average normalized transferrin peak intensity	St. dev.	CV%	Intra-CV%	Inter-CV%
Control	4	5.45	1.42	26	24	69
	7	1.84	1.09	59		
	8	5.81	1.85	32		
	11	2.10	0.65	31		
	12	1.72	0.43	25		
	213	7.15	1.20	17		
	214	1.61	0.09	6		
	217	1.28	0.13	10		
	218	1.41	0.17	12		
	222	6.33	1.60	25		
TM exposed	141	1.85	0.44	24	29	76
	142	6.20	0.80	13		
	145	5.98	1.40	23		
	146	1.66	0.45	27		
	149	1.13	0.47	42		
	355	6.19	1.61	26		
	351	1.18	0.49	42		
	352	1.07	0.24	23		
	356	6.53	3.90	60		
	360	1.38	0.10	7		

respectively) and singly and doubly charged anti-Tfn IgG (*m/z* 144,000 and 72,000, respectively). Very few other peaks were present suggesting that the anti-Tfn IgG were highly specific. An expansion of the *m/z* 71,000 to 79,000 region (Fig. 1C) revealed that the mass difference between the *m/z* 75,000 peak (potentially CDT) and the singly changed transferrin was ~2200 Da.

3.4. Comparative peak statistics

Coefficient of variation values (CV%) were calculated to evaluate the intra- and inter-replicate variance (i.e., within and between biological replicates, respectively, Table 1) of the normalized transferrin peak. Both the intra- and inter-replicate variance observed in the mass spectra of TM-exposed rats was slightly higher than the controls, although for both groups the inter-replicate variance was approximately 2.7-fold higher than the intra-replicate variance. A Student's *t*-test comparison of the mean intensities of the transferrin peak in valid control and TM-exposed mass spectra showed they were not significantly different ($p = 0.89$). The mean intensity of the CDT in TM-exposed rat mass spectra, however, was significantly larger than controls ($p < 0.0005$).

Further statistical analysis of the singly charged CDT peak was performed in ClinProTools based on the area between boundaries *m/z* 73,887 and 75,022 (set manually). The area under the receiver operating characteristic (ROC) curve (AUC) obtained for the peak was 0.72 (Fig. 1D). A classification model produced using ClinProTools' Genetic Algorithm incorporating both the singly (peak boundaries *m/z* 73,887–75,022) and doubly charged (*m/z* 36,963–37,621) CDT with leave-one-out cross validation was found to be 85% accurate with 90% sensitivity (nine out of ten true positive classifications) and 80% specificity (two out of ten false positive classifications) when four or more of the nine replicate mass spectra obtained for each sample were classified as belonging to the TM-exposed group.

3.5. Evaluating the targeting of transferrin using icMS

The mass spectrum obtained when the magnetic bead extraction process detailed in Section 2.3 was performed with the application of 15 μL of binding buffer without rat serum at Step II is presented in Fig. 2A. The spectrum obtained when the anti-Tfn IgG was omitted from Step I and only immobilization buffer (25 μL total volume) was applied to the beads is shown in Fig. 2B. The resulting reflecton mode MALDI-TOF mass spectrum obtained for an in-solution tryptic digest of the magnetic bead eluate, whereby extraction was performed as described in Section 2.3 including the addition of anti-Tfn IgG at Step I and TM-exposed rat serum at Step II, is given in Fig. 2C. Of the 73 detected peaks, nine corresponded to the masses of rat transferrin peptides (SwissProt accession number TRFE_RAT, score = 88 from a threshold of 61, 12.8% amino acid sequence coverage) and eight peaks to rabbit antibody peptides (SwissProt accession number IGHG_RABIT, score = 238 from a threshold of 61, 25.4% amino acid sequence coverage). MS/MS data were collected for five peaks designated with an asterisk and are presented in Supporting Information Fig. S1. Mascot database searching failed to identify any other proteins in the sample with scores above the specified threshold. The two mass spectra obtained following the overnight incubation of serum samples from a control rat in the presence and absence of PNGase F and subsequent magnetic bead fractionation are shown in Fig. 2D.

4. Discussion

In a previous study (Penno et al., 2009a), four 2-DE gel spots containing transferrin were found to be differentially displayed in the sera of rats exposed to 40.5 μg/kg body weight/day of TM in pelleted food for 12 months in comparison with controls. Using a combination of Western and lectin blotting it was shown formerly that the sera of TM-exposed rats contained a population of CDT that was not detectable in the controls (Penno et al., 2009a). This supported the known mechanism of action of both TM and CT as irreversible inhibitors of oligosaccharide biosynthesis in the protein *N*-glycosylation pathway (Jago et al., 1983). Accordingly, CDT is particularly promising candidate biomarker of exposure given it may be directly associated with a toxicant-related effect rather than non-specific indicator of a pathological state. In this present investigation, a total serum transferrin ELISA demonstrated that the overall concentration of serum transferrin, irrespective of the glycosylation state, was not altered by toxin exposure (Fig. 1A). This was not unexpected as unlike many glycoproteins transferrin does not need to be glycosylated in order to be released into circulation from the liver (Yeoh et al., 1984).

The technique of icMS profiling, involving the immobilization of transferrin-specific antibodies to protein G functionalized

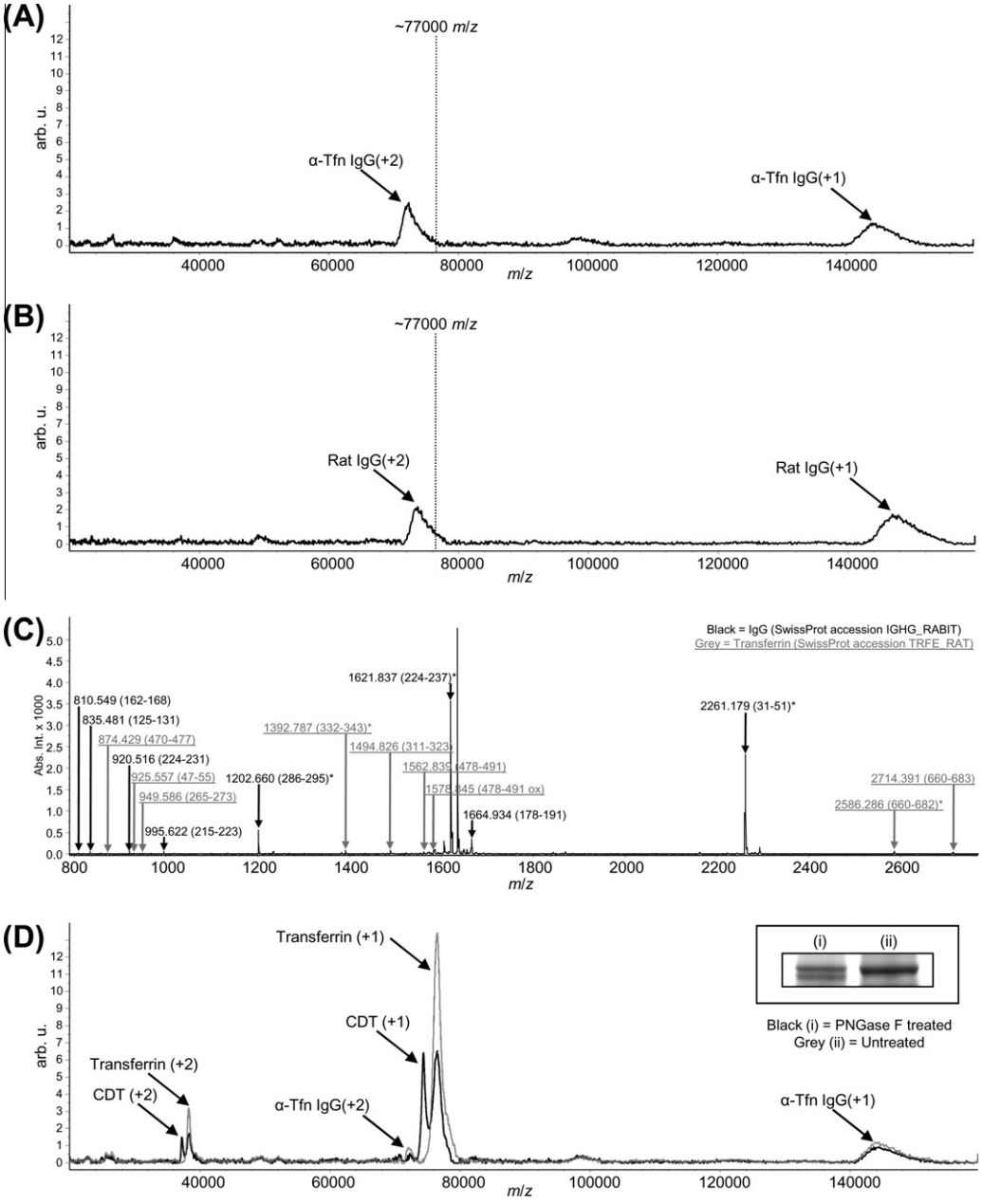

Fig. 2. Mass spectra obtained when the magnetic bead extraction process was performed in the absence of (A) rat serum or (B) anti-Tfn IgG prior to MS analysis. Neither spectrum contained a transferrin peak at *m/z* 77,000. (C) A peptide mass fingerprint spectrum highlighting those peaks corresponding to transferrin and IgG peptides following a tryptic digest of the eluate from magnetic beads treated with the anti-Tfn IgG followed by TM-exposed rat serum. MS/MS spectra were obtained for those peaks indicated with an asterisk. (D) Average mass spectra produced in ClinProTools of control rat serum treated with PNGase F prior to magnetic bead extraction in comparison with an untreated sample. The inset shows the same samples run on an SDS–PAGE gel.

magnetic beads and analysis of the eluate using MALDI-TOF MS, was employed to detect and measure transferrin in rat serum; differentiating between glycosylated transferrin and CDT based on a detectable mass shift. Critically, the serum samples used in this investigation were rapidly frozen in small aliquots after collection and stored at constant −80 °C in monitored freezers until required for the analyses described herein some five years late. Evidence suggests that long-term storage in an effectively maintained ultra-low temperature freezer has a minimal effect on the plasma proteome with no detectable differences in peak numbers, mass distribution, or coefficient of variation values in plasma samples collected four years apart measured by MALDI-TOF MS (Mitchell et al., 2005). A similar finding would be expected for serum. While the degradation of CDT in TM-exposed serum was not measured per se, the average relative intensity of the CDT peak to the main transferrin peak in the mass spectrum rats was 18% (refer to Fig. 1B and C). Densitometric analysis of the CDT band in single dimension SDS–PAGE gels, which were run within 12 months of sample collection, indicated the relative ratio of CDT to main transferrin was 17% (results not shown). Therefore, based on this comparison, the amount of degradation of CDT over time appears to be minimal.

The intra-replicate variation of the mass spectra collected here, which represents the amount of within-sample or technical variance, based on normalized transferrin peak intensity was on average less than 30% (Table 1). This reflects the results of past MS profiling studies (Aivado et al., 2005; Semmes et al., 2005; Albrethsen et al., 2006; de Noo et al., 2006; West-Norager et al.,

2007; Penno et al., 2009b), although it should be noted that these previous investigations involved far lower and narrower mass ranges than the *m/z* 10,000–160,000 used here. The consequences of high intra-replicate variability are that minor yet potential biologically significant differences between physiological states (for example, control versus toxin-exposure) may be overshadowed. Such was not the case here where the average control and TM-exposed mass spectra showed a clearly observable difference with a small peak at *m/z* 75,000 present only in the sera of the TM-exposed rats (Fig. 1B). The mass difference between the singly charged transferrin peak and this *m/z* 75,000 peak corresponded to the mass of the commonly observed non-fucosylated diantennary transferrin glycan known as rTf-3 (Fig. 1C, insert), although the broadness of the *m/z* 77,000 peak could encompass the carbohydrate microheterogenity that is associated with rat transferrin (Spik et al., 1991). Accordingly, the *m/z* 75,000 peak appeared to represent CDT and was consistent with the profile of transferrin and CDT previously observed on the SDS–PAGE gel for the TM-exposed rats (Penno et al., 2009a).

ROC analysis of the CDT peak produced a modest AUC of 0.72 (Fig. 1D). A classification model based on the ClinProTools' Genetic Algorithm however, showed 80% sensitivity and 90% specificity, and overall was 85% accurate at classifying the samples according to their appropriate groups with 17 out of the 20 rats classified correctly. This highlights the ability of an algorithm to improve diagnostic accuracy versus a univariate test centered on a nominal cut-off value, above or below of which is considered as positive or negative. In the absence of an effective assay for detecting CT exposure, whereby the current diagnostic strategy in livestock is based upon non-clinical factors such as the history the affected property and the identification of toxic ryegrass in pastures in association with observable clinical signs of toxicosis that are generally not manifested until an animal is in the terminal stages disease, this is a noteworthy result. Furthermore, the apparent accuracy of the icMS-based assay is consistent with levels reported for other serum protein markers presently used in both veterinary and human medicine, such as the N-terminal pro-B-type natriuretic peptide (NT-proBNP), which is used for the diagnosis of heart failure (Mueller et al., 2005; Oyama et al., 2008).

Four additional experiments were undertaken to confirm that the icMS method was targeting rat transferrin. Firstly, in the absence of rat serum, only the singly and doubly charged peaks corresponding to the anti-Tfn IgG were detectable in the average spectrum (Fig. 2A). The absence of a peak at *m/z* 77,000 indicated that the protein represented by the peak was specifically derived from the serum. Secondly, when serum from a control rat was applied to beads that had been treated with immobilization buffer in the absence of anti-Tfn IgG, the resulting average spectrum contained only two peaks, presumably corresponding to singly and doubly charged rat IgG that would be bound by the protein G beads from the serum (Fig. 2B). A peak at *m/z* 77,000 was not evident in the absence of the anti-Tfn IgG, suggesting that the protein represented by this peak was specifically targeted by the anti-Tfn IgG. Thirdly, a tryptic digest of the magnetic bead elute obtained following the extraction of TM-exposed rat serum using anti-Tfn IgG-treated beads identified several peaks with masses corresponding to both rat transferrin and rabbit IgG peptides (Fig. 2C) with confirmatory MS/MS data obtained for three rabbit IgG and two transferrin peptides (Supporting Information Fig. S1). The fact that the MASCOT search of the combined MS and MS/MS data identified only these two proteins highlights that these were the major components of the sample. Lastly, to support the proposal that the putative CDT ion (*m/z* 75,000) did represent non-glycosylated transferrin, a PNGase F digest of serum from a control rat, which was not expected to contain any endogenous CDT, was subjected to the magnetic bead immunocapture extraction process. As shown in Fig. 2D the *m/z* 75,000 peak was detected in the PNGase F-treated sample but not when serum from the same rat was incubated under identical conditions in the absence of PNGase F. When these samples were analyzed by SDS–PAGE, a transferrin doublet could be seen in the treated but not the untreated serum (Fig. 2D, insert), again consistent with the 1D-E profile of the TM-exposed rats (Penno et al., 2009a).

5. Conclusions

In this investigation, icMS profiling has been used to discriminate between serum samples collected from control rats and rats exposed to dietary TM for 12 months based on the presence of CDT. The icMS approach was found to be very specific with transferrin and IgG representing the largest peaks in the mass spectra and produced a promising classification model. It should be noted that although a single protein was targeted here, icMS can be multiplexed for the simultaneous enrichment of several proteins that could be used to build ion signatures based on multiple peaks (Wang et al., 2008) thereby potentially enhancing the accuracy of the model. Such additional candidate biomarkers of TM exposure could include α_1-microglobulin, α_1-antitrypsin and kininogen 1, which have been identified previously (Penno et al., 2009a). While the practicality of an icMS-based diagnostic assay for disease detection is presently limited by the availability of suitable MS instruments in the clinical laboratory, the increasing popularity of microbial identification by MALDI-TOF MS (Seng et al., 2010) is expected to bring MS-based diagnostics into mainstream clinical usage. Accordingly, the development of this assay represents a significant step towards a high-throughput screening test for detecting CT exposure that may be suitable for both livestock and humans.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.rvsc.2011.08.015.

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