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Toxicogenomic effects of marine brevetoxins in liver and brain of mouse[☆]

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Abstract

Although the polyether brevetoxins (PbTx's) produced by *Karenia brevis* (the organism responsible for blooms of the Florida red tide) are known to exert their acute toxic effects through ion-channel mediated pathways in neural tissue, prior studies have also demonstrated that at least one form of the toxin (PbTx-6) is bound avidly by the aryl hydrocarbon receptor (AhR). Since AhR binding of a prototypical ligand such as dioxin is the first step in a cascade pathway producing major changes in gene expression, we reasoned that PbTx-6 might produce similar genomic-wide changes in expression. Mice were injected i.p. with sub-lethal doses of PbTx's (either 1.5 or 3 mg/g body weight of PbTx-6; or 0.15 mg/g body weight of PbTx-2, a toxin not avidly bound by the AhR), and liver and brain tissues were sampled at 8, 24 and 72 h and RNA was isolated. Changes in gene-specific RNA levels were assessed using commercially available mouse cDNA arrays (Incyte) containing >9600 array elements, including many elements from AhR-mediated genes. Histopathology of the two organs was also assessed. We observed minor histopathological effects and a total of only 29 significant (>2.0-fold) changes in gene expression, most of which occurred in the liver, and most of which could be attributable to an 'acute phase' inflammatory response. These results argue against the hypothesis that PbTx-6 acts via a classic AhR-mediated mechanism to evoke gene expression changes. However, given the avidity with which PbTx-6 binds to the AhR, these findings have important implications for how PbTx's may act in concert with other toxicants that are sensed by the AhR.

Keywords

Marine brevetoxins; Toxic dinoflagellates; *Karenia brevis*; Toxicogenomics; Gene arrays; Metallothioneins; Orosomucoid; Lethal shock

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1. Introduction

Toxins from harmful algal blooms (HABs) in the marine environment are known primarily for their non-genomic effects, with many acting at the level of specific ion channels in the nervous system (either as activators or blockers) or as direct inhibitors of enzymatic reactions (Baden et al., 1995). The marine polyether brevetoxins (PbTx) (Fig. 1), for example, are potent activators of the voltage-gated sodium channel (Jeglitsch et al., 1998; Rein et al., 1994). However, in addition to this acute mode of action, there is some evidence to suggest that some of these toxins are both metabolized and 'sensed' by cells in a manner similar to other toxins with more long-term effects on gene expression (for example, the prototypical aryl hydrocarbon, dioxin). As one indicator of broader effects, brevetoxins have recently been reported to cause developmental abnormalities in fish embryos (Kimm-Brinson and Ramsdell, 2001). Additionally, several studies in fish and mammals have demonstrated that PbTx-3 (an alcohol form of the toxin, Fig. 1) is rapidly cleared from the blood (with a half-life of approx. 30 min) and that the major route of metabolic processing and excretion is via the liver and bile (Cattet and Geraci, 1993; Poli et al., 1990; Kennedy et al., 1992; Washburn et al., 1994; Benson et al., 1999). Furthermore, Washburn et al. (1994) found that PbTx-3 could induce fish liver EROD activity (ethoxyresorufin *O*-deethylase, a cytochrome P450-dependent Phase I detoxification enzyme that oxidizes substrates) by approximately three-fold. In a third study on the effects of metabolic activation, Washburn et al. (1996) examined the effects of a different parent toxin PbTx-2 (an aldehyde form of the toxin, Fig. 1) in fish and again found EROD induction of approximately three-fold. In the same study, examination of the activity of specific liver glutathione transferase (GST) isozymes (π and μ) showed induction by PbTx-2 of 35 and 50%, respectively, (Washburn et al., 1996).

Cytochrome P4501A1-based enzyme activities are induced classically via the aryl hydrocarbon receptor (AhR) binding to an exogenous ligand (e.g. dioxin) in the cytosol, followed by transport to the nucleus by ARNT (Ah receptor nuclear translocator) and interaction with a dioxin response element (DRE) in the promoter region to facilitate transcriptional activation (Landers and Bunce, 1991; Hankinson, 1995). Thus, there is the possibility that PbTx's activate EROD through an AhR-mediated pathway. In this regard, Washburn et al. (1997) examined the ability of the guinea pig AhR to bind various forms of PbTx. As expected from their moderate induction of EROD (compared to for example β -naphthoflavone, BNF), the alcohol and aldehyde forms, PbTx-3 and PbTx-2 were weak binders, and did not readily compete with BNF in gel shift assays; notably, while BNF caused gel shift (indicating binding of BNF-AhR to DRE) in the nanomolar range, only weak shifting was caused by PbTx-2 and -3 in the micromolar range. However, when the naturally occurring epoxide metabolite PbTx-6 (Fig. 1) was used in similar assays, a nearly identical nanomolar response curve to BNF was obtained for PbTx-6 gel shift assays (Washburn et al., 1997).

These findings show that PbTx's are metabolized via the liver and at least one form of the toxin, PbTx-6, can bind to the AhR, suggesting that PbTx's may have more chronic effects possibly at the level of gene expression. To date, there have been no studies of the effects of PbTx-6 on detoxification enzyme induction or more generally there have been no studies of the ability of any of the PbTx's to modulate expression of mRNA from a broad panel of genes. Therefore, the current studies were undertaken with two goals in mind. First, we wished to directly test the hypothesis that PbTx-6 can act at the level of gene expression in the liver to induce a classic, dioxin-like, response of CYP1A1 and related enzymes via an AhR-mediated pathway. The second goal of the study was to more generally explore whether PbTx's had effects at the level of gene expression. To this end, we examined the effects of both PbTx-6 and PbTx-2 in different dosages and after several time periods on global mRNA expression patterns, in both liver and brain of mice. A mouse model was chosen for these initial gene expression studies because of the greater availability of commercial arrays compared to that for aquatic test organisms, as

well as, because of the larger potential for translation of results with mice to biomarkers for human PbTx exposure. Contrary to expectations, our results demonstrate that PbTx's do not appear to act via these classic AhR-mediated pathways, and furthermore, that genomic effects of PbTx's are not widespread and are more consistent with a general 'acute phase response' to toxic agents.

2. Materials and methods

2.1. Toxins

All reagents used were Fisher Scientific (Pittsburg, PA) HPLC-grade for liquids or certified A.C.S. for solids, unless otherwise noted. PbTx-2 was purified from cultures of *Karenia brevis* by a combination of liquid-liquid extraction, flash chromatography, TLC and HPLC using established procedures (Baden et al., 1981). PbTx-6 was prepared, using standard procedures, from PbTx-2 by treatment with dimethyldioxirane followed by HPLC purification (Adam et al., 1987; Rein et al., 1994). In detail, first a three-fingered 100 ml round bottom flask was attached to a condenser via the middle finger, one side finger was set up to receive argon gas and the third finger was used to add reagents. The condenser was attached to a cold finger using a U connector. Dry ice and acetone were added to the cold finger prior to the start of the synthesis. At the bottom of the cold finger was a vacuum connector connected to a 25 ml round bottom receiving flask containing 10 ml acetone, 10 mg PbTx-2 and a stir bar. The receiving flask was immersed in a water bath at 4 °C and continuously stirred. Twelve grams of sodium bicarbonate were added to the three-fingered flask using a powder funnel and then 10 ml ddH₂O was added through the same finger and the mixture was stirred using a magnetic stirrer. The argon gas flow was started and pulled through the system using a slight vacuum. Then 25 g oxone (Aldrich, Milwaukee, WI) was added in four aliquots, 5 min apart, to the aqueous sodium bicarbonate. The reaction was allowed to progress for 1 h and the reaction stopped. The distillate was dried down and purified by HPLC with UV detection (Varian C18 semi-prep column, 0.8×25 cm, mobile phase=90% aqueous methanol, λ =215 nm). Purity of PbTx-6 was 98% or better as determined using HPLC-UV. Toxins were aliquoted and stored under argon in glass ampoules and were dissolved in an appropriate volume of phosphate buffered saline (PBS; Sigma) with 0.02% alkamuls just prior to injection.

2.2. Animals and exposures

Adult female (non-pregnant) breeder mice (BALB/C) were purchased from Harlan (Indianapolis, IN). Animals were group housed in standard shoebox cages and given food and water ad libitum. After 7 days of acclimation, mice were injected intra-peritoneally (i.p.) with either the toxin preparation or control vehicle (described below) with sterile 1 cc syringes and 25 gauge needles. Animals were observed for signs of distress continuously for the first 20 min after injection, and then twice daily until euthanasia. All animal usage was conducted at Mt. Sinai Medical Center, an AALAC accredited facility and approved by that institution's internal animal care and use committee.

Groups of mice were injected i.p. with 300 μ l of PbTx PBS/alkamuls solution (or PBS/alkamuls only for control-sham injected) to achieve a final sub-lethal dose (see Section 3) of either 1.5 mg/g or 3 mg/g of PbTx-6, or 0.15 mg/g of PbTx-2. At various time points (8, 24 and 72 h) PbTx-exposed and control mice were killed by CO₂ inhalation, and liver and brain were removed and placed in RNALater (Ambion) and chilled at -20 °C until RNA extraction. Additionally, a small piece of liver and the whole brain were processed for histopathology (see below). Control and experimental groups and sample sizes were: 8 h controls ($N=4$), 8 h 1.5 μ g/g PbTx-6 ($N=3$); 24 h controls ($N=4$), 24 h 3 μ g/g PbTx-6 ($N=3$), 24 h 0.15 μ g/g PbTx-2 ($N=4$); 72 h controls ($N=4$), 72 h 1.5 μ g/g PbTx-6 ($N=4$).

2.3. Histopathology

Major organs (liver, brain, heart, lung, spleen, kidney and intestines) were harvested and placed in 10% neutral buffered formalin (Sigma), processed and paraffin embedded, and stained with hematoxylin and eosin for examination by light microscopy.

2.4. Tissue extractions, preparation of mRNA/cDNA probes and array hybridizations

Total RNA was isolated from 0.2 g of each tissue of each animal by homogenization in 1.2 ml phenol-guanidinium thiocyanate (Trizol Reagent, Gibco BRL) followed by standard chloroform extraction and isopropanol precipitation (Sambrook et al., 1989). Total RNA from each animal within each time and treatment group was examined quantitatively and qualitatively with an Agilent 2100 Bioanalyzer and then pooled in equal amounts, and mRNA was enriched from total RNA using an mRNA purification kit based on oligo(dT) latex bead binding (Qiagen). Cy3-(control) or Cy5-(PbTx-exposed) labeled cDNA was synthesized using MMLV reverse transcriptase according to manufacturer's specifications (Incyte Genomics), including supplied yeast RNA templates, which spanned the range of -25-+25-fold variations in abundance and which were complementary to specific yeast DNAs printed onto the array. Equal amounts of the two probes in a given pair were mixed and Cy3- and Cy5-labeled probes were separated from unincorporated dye by ChromaSpin columns (Clontech). Labeled cDNA probes were then precipitated and resuspended in buffer prior to hybridization to Mouse UniGem DNA Arrays (MUG 1C, Incyte Genomics). These arrays have >9500 elements, including tens of elements representing genes encoding for enzymes of xenobiotic detoxification expected to be regulated via the AhR. After 6 h hybridization at 60 °C, arrays were washed, dried, and scanned in an Axon GenePix 4000 scanner and analyzed with GenePix Pro software. Two or three arrays were performed for each probe pair for each experimental tissue, time and condition. Dye reversals were not required by the manufacturer's specifications and therefore, were not performed in order to conserve costs.

2.5. Array analysis and statistics

Scanned images were imported into GEMTools software (Incyte Genomics) and analyzed for balanced differential expression changes. We used a global normalization method to eliminate any differences in starting amounts of mRNA or total dye incorporation. In order to qualify for analysis, an array had to pass all Incyte QC tests based on control yeast RNA elements. Elements showing an expression change of greater than 1.74-fold up- or down-regulation in two or more replicate arrays for a given experimental pair were exported to Excel spreadsheets and results were further manipulated in this software. Because the number of elements showing changes in expression was relatively small and manageable by this software, cluster analysis was deemed unnecessary. Instead, simple means and standard errors of gene expression changes for multiple arrays are reported. Annotation was accomplished by manually searching PUBMED database, as well as, by using two web-based gene ontology tools ('Dragon': <http://pevsnerlab.kennedykrieger.org/dragon.htm> and 'onto-express', Khatri et al., 2002: <http://www.openchannelfoundation.org/projects/Onto-Express>).

3. Results

3.1. Histopathology

The dosages of toxin used were sub-lethal. The LD50 for PbTx-2 injected i.p. in mice from our laboratory is 0.35 mg/g (J. Zaia, unpubl. data), so our injected dose of 0.15 mg/g was approximately 40% of this LC50 value; both slightly lower (0.17 mg/g, Baden and Mende, 1982) and slightly higher (0.5 mg/g, Baden, 1989) have been reported. Similarly sub lethal doses of PbTx-6 were used in comparison to its LD50 value which has been reported to be

approximately fivefold higher than that for PbTx-2 (Rein et al., 1994). No mice showed any signs of distress other than momentary discomfort from the actual i.p. injection.

The primary lesions observed in all mice included mild, non-specific degenerative changes (vacuolar degeneration and hepatocellular atrophy) of hepatocytes and mild hemosiderin deposition in liver and spleen. The remaining tissues, including brain, did not contain pathologic changes. There were no significant differences (chi-square test, d.f.=1, $P>0.05$) in the prevalence of hepatocellular degeneration between control and PbTx-injected mice [range=33–100% of mice from all groups; 50 and 30% of control mice (all time points combined) vs. 85 and 69% of PbTx-6 injected mice (all time points combined) for hepatocellular atrophy and vacuolar degeneration, respectively]. Mild hemosiderin deposition was observed in scattered spleen samples from across all treatment groups. The presence of hemosiderin (a hemoglobin degradation product) in liver, however, was observed only in the 72 h time point of the PbTx-6 injected mice (50% of mice vs. none in any other treatment).

3.2. Toxicogenomics

3.2.1. Liver—Across the various treatments, a total of only 29 elements (0.3% of elements analyzed) showed differential mRNA expression in the liver by the criterion of greater than two-fold change on at least two replicate arrays for a given treatment. After 24 h of exposure to 0.15 mg/g PbTx-2, nine genes changed mRNA expression uniquely in response to this toxin (four of which are unidentified) (Table 1). After 8 or 72 h of exposure to PbTx-6, 13 genes changed mRNA expression uniquely in response to this toxin (one of which is unidentified) (Table 2). There were 7 genes (one of which is unidentified), for which expression changes were observed in response to both PbTx-2 and PbTx-6 and these are listed in Table 3. In only one liver sample/array did we observe an increase in expression of mRNA for a xenobiotic detoxification enzyme, namely a +2.4-fold increase in CYP4a14 (accession number AA106365, element location 7910) after 24 h exposure to 3 mg/g PbTx-6, however, this change was not seen in any other sample, nor were any other xenobiotic detoxification enzyme expression changes noted in any samples/arrays. There were two notable gene expression changes just at or below the 2.0-fold cut-off. Namely in four arrays (two each from the 24 h 0.15 mg/g PbTx-2 and the 8 h 1.5 mg/g PbTx-6 treatments) there were changes of −1.9, −2.0 and −1.9, −2.0, respectively, in expression of Inter alpha trypsin inhibitor, heavy chain 4 (accession number AA049060, element location 2308). In the 24 h 0.15 mg/g PbTx-2 treatment there were also +1.9, +1.9 changes in expression of Interleukin-7 (accession number AA416355, element location 1587).

3.2.2. Brain—Changes in gene expression patterns in brain tissue were even more limited than for liver with only nine significant changes being observed for PbTx-2 (and five of these were unknown or showed low homology to identified genes). No significant expression changes by our criteria were observed for PbTx-6 treatment.

4. Discussion

While the acute electrophysiological effects of marine brevetoxins have been very well characterized (Jeglitsch et al., 1998; Rein et al., 1994), the more chronic impacts of brevetoxins on biological systems have been less well studied. The present study aimed to examine specifically the potential effects of brevetoxins on patterns of gene expression. In this study, a single sub lethal dose of either PbTx-2 or PbTx-6 was injected into mice and this dose produced only modest histopathological responses. In fact, there were no statistically significant differences between control and PbTx-injected mice in histopathological parameters (see Section 3). Based on the present histopathological data and biochemical responses of animals to PbTx's observed in prior studies using similar doses (Cattet and Geraci, 1993; Poli et al.,

1990; Kennedy et al., 1992; Washburn et al., 1994, 1996, 1997; Benson et al., 1999), we believe that an appropriate balance was struck in the current study between injecting a dose high enough to elicit any potential responses at the level of gene expression vs. a dose which would have produced severe toxicity or death.

In the livers of mice injected with either PbTx-2 or PbTx-6, we observed significant changes in gene expression in a very low number of genes (only 29 elements, Tables 1–3), compared to a classic dioxin response, where changes in expression of hundreds of genes have been observed in cultured human cells (Puga et al., 2000). Specifically, our results further indicate the lack of a broad-based up regulation of xenobiotic detoxification pathways in response to PbTx exposure, suggesting that the modest increases seen in enzyme activities for cytochrome P450-based oxidases and glutathione-S-transferases (e.g. Washburn et al., 1994, 1996) can be effected by only modest (<1.74-fold) changes in gene expression or alternatively by translational or post-translational mechanisms. Our results (Tables 1–3) also argue against the hypothesis that PbTx-6 or PbTx-2 is acting via a classic AhR-mediated pathway. While these findings are as expected for PbTx-2, which is only weakly bound by the AhR (Washburn et al., 1997), the outcome for PbTx-6, which is avidly bound by the AhR (Washburn et al., 1997), is surprising. Our results suggest that when organisms are exposed to PbTx-6 in nature or if it is produced by metabolism of other forms of PbTx, PbTx-6 may act as a non-effective ligand for AhR receptors. While not directly exerting classical dioxin-like gene level impacts, it is possible that PbTx-6 might act as a modifier of these responses by, for example, interfering with binding of other effector ligands. Thus, we would predict that, when PbTx-6 is a co-toxicant with other AhR-binding toxins, it may act to blunt the gene expression response to the second toxicant. This mode of action might be particularly problematic and chronic if, as studies suggest (e.g. Kennedy et al., 1992) that a certain fraction of PbTx remains in a deep body compartment.

Despite the absence of broadly based gene expression changes in liver to PbTx, there are some interesting genomic effects in this organ. The majority of the gene expression changes (those denoted with a superscript 'a' in Tables 1–3 and the two marginally significant changes noted in Section 3 above) are consistent with an acute phase inflammatory response to a toxic agent (van Molle et al., 2000; Uwe et al., 2002; Pagano et al., 2002; Rosenberg, 2002; Chen et al., 1997; Yousefi and Simon, 2002; Escriba et al., 2002; Chui et al., 2002; Wielockx et al., 2001). Notably, our studies reinforce the recent findings of Waelput et al. (2001) that metallothioneins are involved in mediating a down-regulation of this response (Table 3) presumably to prevent the potentially lethal systemic inflammatory response syndrome.

It should be noted that although the PbTx-induced responses obtained in this study were achieved via parenteral administration, in the natural setting, PbTxs can be aerosolized and so the lungs can become a major route of exposure. Recent studies in a sheep model of allergic asthma show that inhaled PbTx-2 and PbTx-3 produce acute bronchoconstriction (Singer et al., 1998; Abraham and Baden, 2001; Abraham et al., 2002). The mechanism for the bronchial response in the sheep model appears to involve, not only cholinergic stimulation (Singer et al., 1998) as indicated in previous studies in other species (Baden et al., 1982; Baden, 1989; Watanabe et al., 1988), but the stimulation of airway mast cells and the local release of bronchoactive and inflammatory mediators (Abraham and Baden, 2001; Abraham et al., 2002). In spite of these local responses, however, it appears that these inhaled toxins are rapidly cleared from the lungs and blood and the liver becomes a major route of metabolic processing (Benson et al., 1999). Whether or not chronic exposure to inhaled PbTxs would induce similar changes in liver gene expression has not been determined, but given the local inflammatory response, would be an important study.

Several other changes in liver gene expression in response to PbTx are difficult to explain currently. Mouse major urinary protein (Table 2) binds to pheromones in mouse urine that are used in a variety of social behaviors, and thus would not appear to be linked to an acute phase response (Timm et al., 2001). However, the urinary protein is a member of the lipocalin family involved in the acute phase response (Ferrari et al., 1997), where we did see a change in expression (Table 2) so perhaps the regulation of urinary protein and lipocalin are linked via common gene responsive elements. Neural cell adhesion molecule (Tables 1 and 3) is associated with neuronal growth, and if these changes were observed in brain, one could make the logical interpretation that the proteins were contributing to regeneration of cells damaged by PbTx (Thelan et al., 2002; Murase and Schuman, 1999). The role of neural cell adhesion molecule up regulation in liver remains unclear. Betaine-homocysteine methyltransferase (Table 2) plays a role in several metabolic pathways of glycolipid transformations, and also is a zinc metalloenzyme (Breska and Garrow, 2002) and does not appear to have an obvious connection to PbTx or acute phase response.

The results for brain gene expression patterns further confirm that PbTx appears to exert its effects acutely via ion channels in this target tissue. The few gene expression changes observed (Table 4) do not really appear to be interrelated. For example, Xist is believed to play a role in developmental suppression of one X chromosome (Brockdorff, 2002) and Mest is believed to be linked to early imprinting (Nishita et al., 1996).

Our results permit the overall conclusion that at the dosages, times and exposure methods used, PbTx's are not acting by widespread AhR-mediated changes in gene expression as one would see with a more classic AhR-sensed toxicant like dioxin. However, when taken together with previous findings on the avidity with which AhR binds PbTx-6, we predict that PbTx's may adversely impact an organism's ability to respond to other AhR-sensed toxicants and thus the chronic toxicity of PbTx should be further assessed in toxicant mixtures. A final caveat is called for relative to potential PbTx genomic effects in aquatic organisms: since near-shore marine and estuarine organisms still encounter red tides, their responses to these toxins may be more finely tuned and pronounced relative to test subjects such as mice. Thus, it may be fruitful to examine genomic effects of PbTx's in such aquatic organisms for which micro arrays are now becoming available (e.g. the common killifish, *Fundulus heteroclitus*) (Oleksiak et al., 2002).

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Fig. 1.
Structures of three common forms of the brevetoxin (PbTx) molecules.

Table 1

Changes in gene expression in mouse liver following 24 h exposure to 0.15 mg/g PbTx-2

Element location	Balanced differential expression	Gene name	Accession number
1354	+2.1, +2.1 (2)	ESTs	W18499
1613	+2.1, +2.1 (2)	Troponin I, skeletal, fast 2	AA422743
2077	+2.1±0.1 (3)	ESTs	AA822370
3809	+2.1, +2.1 (2)	Neural cell adhesion molecule	W29590
6538 ^a	+2.2±0.1 (3)	ESTs (Mouse apolipoprotein ApoA-1/Apoc-3)	AA254866 (MUSAICIII A)
8823	-2.4±0.1 (3)	ESTs	AI591847
8992	+2.1, +2.1 (2)	RIKEN cDNA 2810054M15 gene	AI645238
9720	+2.2±0.1 (3)	Public domain EST	
9851 ^a	+2.2±0.1 (3)	Tissue inhibitor of metalloproteinase 3	AI197159

Values are mean ± S.E. (*N*) if *N*>2, otherwise replicate values are listed. Gene name and accession number in parentheses were discovered by BLAST analysis of the EST sequence in GenBank and provide a likely identity to the EST.

^a Indicates involvement in acute phase response, see text for details.

Table 2

Changes in gene expression in mouse liver following exposure to 1.5 mg/g PbTx-6

Element location	Balanced differential expression	Gene name	Accession number
8 h			
254	-2.1, -2.1 (2)	RIKEN cDNA 1110032A13 gene	AA105771
581 ^a	-2.3, -2.2 (2)	Serine protease inhibitor 2-1	AA727334
1264 ^a	-2.2, -2.1 (2)	Kallikrein binding protein	W14912
3032 ^a	-2.1, -2.1 (2)	Serine protease inhibitor-2 related sequence 1	AA733490
3034	-2.2, -2.2 (2)	Major urinary protein 2	AA822105
4939	-2.5, -2.6 (2)	Betaine-homocysteine methyltransferase	AA272831
5061	+2.3±0.3 (3)	Moloney leukemia virus 10	W34188
7947 ^a	-2.5, -2.5 (2)	Apolipoprotein E	AI325603
8135 ^a	-2.1, -2.1 (2)	Serine protease inhibitor 2-2	AA821980
8147 ^a	-2.4, -2.5 (2)	Apolipoprotein A-I	AA822098
9199	+2.1, +2.1 (2)	RIKEN cDNA 1110038L14 gene (Cyclin-dependent kinases reg. subunit 2)	AA413740 (AK004163.1)
8 h and 72 h			
2089 ^a	-4.3±1.8 (3)	Serum amyloid A 3	AA881525
3728 ^a	-3.7±1.5 (3)	Lipocalin 2	AA087193

Values are mean ± S.E. (*N*) if *N*>2, otherwise replicate values are listed. Genes that showed altered expression in only arrays of 8 h exposure are grouped separately from those, which also showed altered expression in a 72 h exposure. Gene name and accession number in parentheses were discovered by BLAST analysis of the EST sequence in GenBank and provide a likely identity to the EST.

^aIndicates involvement in acute phase response, see text for details.

Table 3

Changes in gene expression in mouse liver following exposure to 1.5 mg/g (8 and 72 h), 3 mg/g (24 h) PbTx-6 and 0.15 mg/g PbTx-2 (24 h)

Element Location	Balanced differential expression	Gene name	Accession number
	8 h, 24 h and 72 h		
5043 ^a	-2.5±0.2 (4)	Metallothionein 2	W36474
8141 ^a	-2.3±0.1 (5)	Hemopexin	AA822009
8665 ^a	-2.6±0.1 (6)	Metallothionein 1	AA638765
9488	-2.8±0.2 (6)	RIKEN cDNA 2900017D14 gene	AA684321
4903 ^a	-2.5±0.6 (3)	Orosomucoid 2 (alpha 1-acid glycoprotein)	AA245687
	24 h		
2944	+2.7±0.4 (3)	ESTs (Putative neuronal cell adhesion molecule (Punc).gene)	AA108788 (AF026466)
8381	+3.2±0.1 (5)	ESTs (Protein phosphatase 2C Alpha 3)	AI447967 (AF259672)

Values are mean ± S.E. (N) and each mean contains at least one array from either toxin exposure. Genes that showed altered expression in only arrays of 24 h exposure are grouped separately from those, which also showed altered expression in 8 and 72 h exposures. Gene names and accession numbers in parentheses were discovered by BLAST analysis of the EST sequence in GenBank and provide a likely identity to the EST.

^a Indicates involvement in acute phase response, see text for details.

Table 4

Changes in gene expression in mouse brain following exposure to 0.15 mg/g PbTx-2

Element location	Balanced differential expression	Gene name	Accession number
	8 h		
1901	-2.4, -2.2	ESTs, Highly similar to phosphodiesterase I [<i>R. norvegicus</i>]	AA871395
2107	-2.3, -2.1	Homeo box A2	AA929366
6724	-2.3, -2.2	ESTs, moderately similar to T34531 hypothetical protein DKFZp434P1215.1 [<i>H. sapiens</i>]	AA437717
6933	+2.3, +2.2	ESTs	AA684403
7093	-2.6, -2.4	ESTs (<i>Mus musculus</i> X-inactivation center region (Xist gene))	AA690387 (AJ421479.1)
7417	-2.3, -2.1	Public domain EST	
7695	-2.2, -2.1	ESTs, weakly similar to T30021 hypothetical protein K08F11.4 [<i>C. elegans</i>]	AI536305
9488	-5.8, -4.3	RIKEN cDNA 2900017D14 gene	AA684321
	24 h		
9644	-2.2, -2.1	Mest-linked imprinted transcript 1	W57281

Values are duplicates. Genes that showed altered expression in only arrays of 8 h exposure are grouped separately from that which also showed altered expression in 24 h exposure. Gene names and accession numbers in parentheses were discovered by BLAST analysis of the EST sequence in GenBank and provide a likely identity to the EST.