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Transcriptomic profile of host response in mouse brain after exposure to plant toxin abrin

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ABSTRACT

Abrin toxin is a plant glycoprotein, which is similar in structure and properties to ricin and is obtained from the seeds of *Abrus precatorius* (jequirity bean). Abrin is highly toxic, with an estimated human fatal dose of 0.1–1 μg/kg, and has caused death after accidental and intentional poisoning. Abrin is a potent biological toxin warfare agent. There are no chemical antidotes available against the toxin. Neurological symptoms like delirium, hallucinations, reduced consciousness and generalized seizures were reported in human poisoning cases. Death of a patient with symptoms of acute demyelinating encephalopathy with gastrointestinal bleeding due to ingestion of abrin seeds was reported in India. The aim of this study was to examine both dose and time-dependent transcriptional responses induced by abrin in the adult mouse brain. Mice ($n = 6$) were exposed to 1 and 2 LD50 (2.83 and 5.66 μg/kg respectively) dose of abrin by intraperitoneal route and observed over 3 days. A subset of animals ($n = 3$) were sacrificed at 1 and 2 day intervals for microarray and histopathology analysis. None of the 2 LD50 exposed animals survived till 3 days. The histopathological analysis showed the severe damage in brain and the infiltration of inflammatory cells in a dose and time dependent manner. The abrin exposure resulted in the induction of rapid immune and inflammatory response in brain. Clinical biochemistry parameters like lactate dehydrogenase, aspartate aminotransferase, urea and creatinine showed significant increase at 2-day 2 LD50 exposure. The whole genome microarray data revealed the significant regulation of various pathways like MAPK pathway, cytokine-cytokine receptor interaction, calcium signaling pathway, Jak-STAT signaling pathway and natural killer cell mediated toxicity. The comparison of differential gene expression at both the doses showed dose dependent effects of abrin toxicity. The real-time qRT-PCR analysis of selected genes supported the microarray data. This is the first report on host-gene response using whole genome microarray in an animal model after abrin exposure. The data generated provides leads for developing suitable medical counter measures against abrin poisoning.

1. Introduction

Ribosome-inactivating proteins (RIPs) are a group of proteins that share the property of damaging ribosomes in an irreversible manner acting catalytically (Stirpe, 2004). Examples of plant RIPs include abrin, ricin, gelonin, momordin, mistletoe lectin, etc. (Narayanan et al., 2005). Abrin is a heterodimeric glycoprotein found in the beans of *Abrus precatorius* plant commonly known as jequirity bean or rosary pea. Abrin and ricin are structurally and functionally related protein toxins. Toxic protein abrin and ricin are among the most poisonous substances known only next to botulinum toxin. Abrin is a potent biological warfare agent because of its low cost of isolation and ease of use either by aerosolisation as a dry powder or liquid droplets, or by addition to food and water as a contaminant.

Abrin belongs to the family of type II ribosome inactivating proteins (RIPs) comprising of A and B subunits cross-linked by a single disulfide bond. The active A-chain moiety has an enzymatic function. The toxicity of A-chain is due to its RNA-N-glycosidase activity, by which it brings about depurination of adenine at 4324 in the 28S rRNA. The end result of this activity is complete inhibition of cellular translation (Irvin, 1995). Polypeptide B chain (38 kDa) is a galactose-specific lectin that facilitates the binding of abrin to cell membranes, while the other chain A enters the cytoplasm. Once in

Abbreviations: CNS, central nervous system; GSH, glutathione; PBS, phosphate buffered saline; qRT-PCR, quantitative real-time polymerase chain reaction; MAPK, mitogen activated protein kinase; KEGG, Kyoto Encyclopedia of Genes and Genomes; DAVID, Database for Annotation, Visualization and Integrated Discovery.

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the cell, the A chain acts on the 60S ribosomal subunit, preventing binding of elongation factor 2, thus inhibiting protein synthesis and leading to cell death (Sandvig et al., 1976; Irvin, 1995). Though RIPs were identified more than few decades ago, there is still speculation about their biological functions. Recent studies have shown clearly RNAse and DNAse activities of RIPs including ricin and abrin (Barbieri et al., 1997, 2000). There are recent reports on the DNA damage effects of RIPs like ricin and (Rao et al., 2005) abrin (Shih et al., 2001; Bhaskar et al., 2008).

Abrin is highly toxic, with an estimated human fatal dose of 0.1–1 μg/kg, and has caused death after accidental and intentional poisoning. Abrin can be extracted from jequirity beans using a relatively simple and cheap procedure. This satisfies one criterion of a potential chemical warfare agent, although the lack of large-scale production of jequirity seeds means that quantity is unavailable for ready mass production of abrin for weapons (Dickers et al., 2003).

There are several reported cases of ricin poisoning and few cases of abrin poisonings (Rauber and Heard, 1985). A number of poisoning cases due to abrin were reported from India (Sahni et al., 2007; Subrahmanyan et al., 2008; Sahoo et al., 2008; Pillay et al., 2005). Clinical features of abrin poisoning commonly include nausea, vomiting, diarrhea, and abdominal pain. Gastrointestinal bleeding may ensue, with bloody diarrhea (Davis, 1978; Frohne et al., 1984) and/or hematemesis (Dickers et al., 2003). Neurological symptoms like delirium, hallucinations, reduced consciousness and generalized seizures were reported (Frohne et al., 1984). Death of a patient with symptoms of acute demyelinating encephalopathy with gastrointestinal (GI) bleeding due to ingestion of abrin seeds was reported in India (Sahni et al., 2007; Sahoo et al., 2008).

The measurement of gene expression levels upon exposure to a chemical can both provide information about the mechanism of action of toxicants and signature pattern of expression (Hamadeh et al., 2002; Lettieri, 2006). Toxicogenomics aim to apply both mRNA and protein expression technology to study chemical effects in biological systems. The development of high quality, commercially available gene arrays has allowed this technology to become a standard tool in molecular toxicology. No reports are available on whole genome array after exposure to abrin.

However, there are few reports on genomic expression profiles of another RIP ricin whose mode of action is similar to abrin. The pulmonary genomic profile of BALB/c mice inhalationally exposed to lethal dose of ricin was examined using cDNA arrays (DaSilva et al., 2003). cDNA array results have identified a number of differentially expressed genes responsible for a variety of activities, such as inflammatory processes, tissue and DNA repair, cell migration and structure, cell growth and differentiation and apoptosis (DaSilva et al., 2003).

The present study is thus designed to understand the consequences and the molecular mechanism of abrin toxicity in CNS using cDNA array technology. Swiss albino mice were exposed to 1 (2.83 μg/kg) and 2 LD50 dose of abrin (5.66 μg/kg body weight) by intraperitoneal route. Dose and time dependent effects of abrin on gene expression profile of brain tissue were examined using mouse whole genome array. Considering the number of neurological symptoms reported in poisoning cases, brain was selected for gene expression analysis in the present study. The differential genomic profile correlated with the pathological changes observed in the brain, with the appearance of indicators of inflammation, and an increase in infiltrating leukocytes. The abrin exposure resulted in the induction of a rapid immune and inflammatory response in brain. The comparison of differential gene expression at both the doses showed dose dependent effects of abrin toxicity. The real-time qRT-PCR analysis of few selected genes supported the microarray data. This is the first report of gene expression profiling after abrin exposure.

2. Materials and methods

2.1. Animals

Swiss albino mice randomly bred in Institute's animal facility, weighing between 24 and 26 g were used in this study. The animals were maintained on standard conditions of temperature and humidity. The animals were fed standard pellet diet (Ashirwad Brand, Chandigarh, India). Food and water were given ad libitum. The animals were handled according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) and Institutional Animal Ethics Committee approved the experiment.

2.2. Chemicals

Abrin was isolated from *A. precatorius* seeds as described elsewhere (Kumar et al., 2008). The purified abrin was lyophilized and stored at −80 °C and reconstituted as and when required in PBS. The whole genome mouse 4x44K microarrays and RNA 6000 Nano Lab Chip were purchased from Agilent (Germany) and processed at Genotypic Technology (Bangalore, India). Low RNA Input Fluorescent Linear Amplification Kit was purchased from Agilent (Santa Clara, CA). QIAamp viral RNA mini kit, total RNA extraction kit, RNAlater, Quanti Tect primer assay kit and Quanti Fast one-step real time RT-PCR kit were purchased from Qiagen (Hilden, Germany).

2.3. Animal exposure

Animals were divided into three groups of six animals each. Groups 1 and 2 were administered a single dose of 1.0 and 2.0 LD50 of abrin (2.83 and 5.66 μg/kg body weight respectively) by intraperitoneal route. Three animals from each group were killed at day 1 and day 2 to harvest brain tissue. The animals were perfused with cold PBS before harvesting the brain tissue. Control animals received the same volume of PBS as the experimental group.

2.4. Biochemical assays

The clinical biochemical parameters were assessed in a separate study with six mice for each treatment group (1 and 2 LD50) and time point (1 and 2 days). For biochemical studies, blood was drawn from orbital plexus before sacrificing the animals. Serum harvested from each mouse was used to determine the biochemical markers alanine aminotransferase (ALT), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), urea, uric acid, alkaline phosphatase (ALP), creatinine and albumin. Serum variables were measured using commercially available diagnostic kits following manufacturer's protocol.

2.5. Sample acquisition, RNA isolation and quality control

Brain tissues were collected from control and treated groups of mice at different days of post-exposure. The sections of cerebral cortex of brain tissue were stored in RNA*later* (Qiagen, Hilden, Germany) at −70 °C until processed for RNA extraction. Total RNA was extracted using the Qiagen (GmbH, Hilden) RNEasy® Mini kit according to the instructions of the manufacturer. RNA quality and integrity was assessed using RNA 6000 Nano Lab Chip on the 2100 Bioanalyzer (Agilent, Germany) following the manufacturer's protocol. RNA samples with RIN (RNA Integrity Number) ≥8 were used in all experiments. The pooled RNA samples were used for microarray analysis to reduce the effects of biological variation and to easily find the substantive differences (Kendziorski et al., 2005). However, individual RNA was also verified with real time RT-PCR without pooling and no significant variation in gene expression was observed.

2.6. cDNA microarrays and hybridization

Low RNA Input Fluorescent Linear Amplification Kit (Agilent, Santa Clara, CA) was used for labeling. Briefly, both first and second strand cDNA were synthesized by incubating 500 ng of pooled total RNA with 1.2 μl of oligo dT-T7 promoter primer in nuclease-free water at 65 °C for 10 min followed by incubation with 4.0 μl of 5× first strand buffer, 2 μl of 0.1 M DTT, 1 μl of 10 mM dNTP mix, 1 μl of 200 U/μl MMLV-RT, and 0.5 μl of 40 U/μl RNaseOUT, at 40 °C for 2 h. Immediately following cDNA synthesis, the reaction mixture was incubated with 2.4 μl of 10 mM Cyanine-3-CTP or 2.4 μl of 10 mM Cyanine-5-CTP (Perkin-Elmer, Boston, MA), 20 μl of 4× Transcription buffer, 8 μl of NTP mixture, 6 μl of 0.1 M DTT, 0.5 μl of RNaseOUT, 0.6 μl of inorganic pyrophosphatase, 0.8 μl of T7 RNA polymerase, and 15.3 μl of nuclease-free water at 40 °C for 2 h. Qiagen's RNeasy mini spin columns were used for purifying amplified cRNA samples. The quantity and specific activity of cRNA was determined by using NanoDrop ND-1000 UV-VIS Spectrophotometer version 3.2.1. Samples with specific activity >8 were used for hybridization. 825 ng of each Cyanine 3 or Cyanine 5 labeled cRNA in a volume of 41.8 μl were combined with 11 μl of 10× blocking agent and 2.2 μl of 25× fragmentation buffer (Agilent), and incubated at 60 °C for 30 min in dark. The fragmented cRNA was mixed with 55 μl of 2× hybridization buffer (Agilent). About 110 μl of the resulting mixture was applied to the Agilent Whole Genome Mouse 4X 44k Gene Expression Microarray (AMADID: 14868, Agilent Technologies) and hybridized in a two-color comparative format at