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Increased expression of immune modulator proteins and decreased expression of apolipoprotein A-1 and haptoglobin in blood plasma of sarin exposed rats

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ABSTRACT

Sarin is a highly toxic organophosphonate and neural enzyme acetylcholinesterase (AChE) inhibitor. Inhibition of AChE causes large accumulation of acetylcholine at synaptic cleft leading to hyper activation of nicotinic and muscarinic acetylcholine receptors, causing excessive secretions, muscle fasciculation, nausea, vomiting, respiratory distress and neurological effects. There are cases in which long term psychomotor function deficiency, reduced learning and memory functions have been observed several years after exposure of sarin among survivors. This phenomenon is called Organophosphorus ester Induced Chronic Neurotoxicity (OPICN) and cannot be explained by AChE inhibition alone. Plasma proteomics at earlier stages was carried out to study changes reflected at blood level that can help predict possible neurological insults at an early time point to take proper therapeutic interventions against OPICN.

In the present study, a 0.5 LD_{50} dose of sarin was administered to Wistar rats and possible changes in blood plasma proteomic profile were investigated after one and seven days of sarin exposure. Proteins were separated on 2-dimensional gel electrophoresis and identified by MALDI-TOF/MS. Expression profile of major proteins was validated by Western blot. Result showed that after exposure of sarin inhibition of AChE persisted after one week of exposure. There were 14 plasma proteins that showed significant changes in expression (>1.5-fold). It included proteins related to immune function, neurodegenerative condition and chaperone function. Interestingly sarin exposure caused decreased expression of plasma Apolipoprotein A-1 and Haptoglobin on day seven, which are the putative early molecular markers for cognitive impairment and neurodegenerative changes.

1. Introduction

Sarin is an anticholinesterase organophosphonate nerve agent and has been classified as a chemical warfare agent [1,2]. Anticholinesterases exert their toxic manifestation by the inhibition of neural enzyme acetylcholinesterase (AChE). Inhibition of Central nervous system (CNS) AChE leads to large accumulation of acetylcholine at the synaptic cleft [3]. This large build up of acetylcholine at cholinergic synapses and neuro-muscular junctions causes over stimulation of nicotinic and muscarinic acetylcholine receptors leading to cholinergic symptoms like lacrimation, salivation, seizures, fasciculation, tremor and hypothermia [4,1]. Apart from acute toxic effects, sarin also induce long term toxic effect i.e. Organophosphorus Ester Induced Chronic Neurotoxicity (OPICN) when exposed at sub-lethal level [5]. Immediate administration of the anti-muscarinic drug atropine along with an oxime reactivator can counter acute toxic effects of sarin and increases survival but long term neurologic damage cannot be reversed using this treatment [6].

There have been instances when sarin was used by terrorists against civilian populations. First incident occurred in Matsumoto city, Japan, in 1994; where sarin was released in residential area [7]. Another incident was reported in Tokyo subway in March 1995. In these incidents several people died and many residents,

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passengers, rescue workers and police personnel were exposed to sarin [8,9]. Apart from immediate toxic manifestations, several persons including rescue workers, who were less exposed to sarin showed long term psycho-motor function deficiency, neuro-behavioral changes, deficiencies in memory and cognitive functions even after seven years post incidence [10]. Persian Gulf War related illness has been reported among war veterans, who were believed to be exposed to low levels of organophosphorus chemical warfare agents, carbamates and several other compounds [11].

Sarin induced neurological changes are the consequences of sarin induced seizures that cause increased blood-brain-barrier (BBB) permeability, diffuse neuronal cell death in cerebral cortex and hippocampus as well as degeneration of Purkinje neurons in cerebellum [12].

Involvement of non-cholinergic targets in the development neurotoxicity by sarin has also been demonstrated [13,14]. Changes in brain multiple signaling pathways, calcium signaling, acetylcholinesterase gene expression may also contribute to the development of organophosphorus-ester induced chronic neurotoxicity [1,15,16].

In another study, it has been demonstrated that exposure of sarin leads to selective down regulation of genes of acetylcholine synthesis and packaging, that includes vesicular acetylcholine transporter (VAChT) and the same had been implicated in impairment of higher cognitive functions during sarin neuro toxicity [2].

Recently, Meade et al. [17] demonstrated that guinea pigs exposed to high doses of sarin showed immediate gross changes in brain stem proteomic profile. These changes include glutamate mediated excitotoxicity, calcium overload, reactive oxygen species (ROS) response and cell death response. These observations lead to the conclusion that there are several early changes that may lead to long term neurologic effects including neurodegeneration.

Blood plasma is the least invasive and readily available source for monitoring of various disease specific biomarkers including Alzheimer's disease [18], schizophrenia [19] and several other neurodegenerative disorders. Apart from major proteins, plasma also contains minor proteins. Expression profile of these minor proteins changes depending on the disease conditions [20]. Cancer and autoimmune disorders are the conditions in which, several plasma protein biomarkers have been identified using LC-MS/MS and/or MALDI-TOF/MS. Plasma contain large amount of high abundant proteins that make the proteomic identification of minor proteins difficult. Expression of these minor proteins fluctuates with pathological conditions. Albumin constitutes about 60% of the plasma proteins which specifically hinders the low abundance protein identification. For analysis of low abundance proteins albumin is first depleted to get enhanced signal of minor proteins [21].

A comparison between the expression profiles of plasma proteins from sarin exposed and control samples would give a greater insight about the changes that had taken place at cellular and molecular level. Moreover, various studies have demonstrated that change in transcriptional profile of mRNA may not always correlate with translational profile, making proteomic analysis a relevant tool for identification of molecular pathology [22].

There is a paucity of literature on plasma proteomic profile after nerve agent exposure. To investigate organophosphorus nerve agent exposure, presently plasma cholinesterase and erythrocyte membrane associated acetyl cholinesterase are estimated as biomarkers of exposure. In the present study we have used proteomic approach to investigate alteration in plasma protein profile after sarin administration to rats. As plasma is quickly and easily available source after exposure, plasma proteomic analysis is expected to give a greater insight into possible neurodegenerative marker protein identification after sarin exposure.

2. Materials and methods

2.1. Chemicals

Sarin (>99% pure) was obtained from the Process Technology Development Division, Defence Research and Development Establishment, Gwalior, India. Its purity was checked by Gas Chromatography and NMR. All reagents required for 2-dimensional gel electrophoresis (2DE) including Immobilized pH gradient (IPG) strips (5–8 pH range) were purchased from M/s BioRad, USA and other chemicals used were of proteomic grade. Anti- Haptoglobin (Hp), Anti- Apolipoprotein A-1 (Apo A-1), Anti-β-Actin antibody and all HRP conjugated secondary antibodies along with other molecular biology grade chemicals were procured from M/s Sigma Chemicals Co. (St. Louis, U.S.A.).

2.2. Treatment of animals

Male Wistar rats (7–8 weeks old), weighing 100–120 g m were maintained and provided by Animal Facilities Division of DRDE, Gwalior. All animal experimental procedures were approved by the Institutional Animal Ethics Committee (No: 37/1999/CPC-SEA). After bringing from Animal Facilities all the rats were housed individually in polycarbonate cages; food and drinking water were given *ad libitum*. Animals were acclimatized for one week in animal room at 21–24 °C with 12 h light–dark cycle. Proper care and maintenance of the animals were taken up according to the proper guide lines of the committee (CPC-SEA, India). Due to slow detoxification of sarin, when injected through subcutaneous route, long term effect of nerve agent can be studied. Hence, subcutaneous route of administration was preferred which is often considered as a reasonable substitute along with intravenous route or respiratory exposure in experimental studies against nerve agent poisoning [1,23,24].

Sarin was always diluted for required dose level in the Institution's Facility for handling toxic chemicals. For this appropriate Face masks and fume hood with scrubber system were used. In experimental group ($n = 10$ for each time point i.e. 2.5hr, 1, 3, 5 and 7th day) rats were injected with single dose of sarin i.e. 80 μg/kg of rats (0.5 LD_{50}) and the control group received 0.9% normal saline by the same route. The subcutaneous LD_{50} of sarin in rats was determined in our laboratory using Dixon's up and down method [25] and the value was 160 μg/kg of body weight.

2.3. Estimation of plasma cholinesterase (ChE) activity

After termination of each time point animals were anesthetized by using anesthetic ether and blood was drawn with the help of heparinized capillary tubes from orbital sinus. Blood was collected in a tube containing anticoagulant. ChE activity was assayed using the method of Ellman et al. [26], as described by Steck and Kant [27]. Acetylthiocholine iodide was used as the substrate. The enzyme activity was expressed as nmol substrate hydrolyzed min^{-1} ml^{-1} of plasma.

2.4. Depletion of albumin and IgG

Albumin and immunoglobin (IgG) are the major constituents of plasma proteins [28] and this need to be selectively removed for the identification of low abundance proteins. Albumin and IgG depletion was done by using Auram serum protein mini kit (BioRad, USA). Briefly, 60 μl of plasma sample was mixed in 180 μl of Aurum serum binding buffer. This sample (200 μl) was loaded on dry resin and it was incubated for 15 min by mixing after every 5 min. Finally column was set in elute tube and centrifuged at 10,000x g for 20 s,