Toxicology and Applied Pharmacology 310 (2016) 87–97

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Propagation of damage in the rat brain following sarin exposure: Differential progression of early processes

Shlomi Lazar, Inbal Egoz, Rachel Brandeis, Shira Chapman, Eugenia Bloch-Shilderman, Ettie Grauer *

Department of Pharmacology, Israel Institute for Biological Research, Ness Ziona, Israel

ARTICLE INFO

Article history:
Received 3 August 2016
Revised 7 September 2016
Accepted 12 September 2016
Available online 14 September 2016

Keywords:
Sarin
Apoptosis
Necrosis
Inflammation
Brain Damage

ABSTRACT

Sarin is an irreversible organophosphate cholinesterase inhibitor and a highly toxic warfare agent. Following the overt, dose-dependent signs (e.g. tremor, hyper secretion, seizures, respiratory depression and eventually death), brain damage is often reported. The goal of the present study was to characterize the early histopathological and biochemical events leading to this damage. Rats were exposed to 1LD50 of sarin (80 μg/kg, i.m.). Brains were removed at 1, 2, 6, 24 and 48 h and processed for analysis. Results showed that TSPO (translocator protein) mRNA increased at 6 h post exposure while TSPO receptor density increased only at 24 h. In all brain regions tested, bax mRNA decreased 1 h post exposure followed by an increase 24 h later, with only minor increase in bcl2 mRNA. At this time point a decrease was seen in both anti-apoptotic protein Bcl2 and pro-apoptotic Bax, followed by a time and region specific increase in Bax. An immediate elevation in ERK1/2 activity with no change in JNK may indicate an endogenous "first response" mechanism used to attenuate the forthcoming apoptosis. The time dependent increase in the severity of brain damage included an early bi-phasic activation of astrocytes, a sharp decrease in intact neuronal cells, a time dependent reduction in MAP2 and up to 15% of apoptosis. Thus, neuronal death is mostly due to necrosis and severe astrocytosis. The data suggests that timing of possible treatments should be determined by early events following exposure. For example, the biphasic changes in astrocytes activity indicate a possible beneficial effects of delayed anti-inflammatory intervention.

1. Introduction

Organophosphate (OP) nerve agents such as soman, sarin, cyclosarin or VX are irreversible potent inhibitors of cholinesterase (ChE). The resulted hyper-cholinergic activity includes salivation, fasciculation, tremors, convulsions, respiratory distress and death (Munro et al., 1994). OP nerve agents' exposure also induce a dose-dependent, wide spread brain damage that typically includes the piriform cortex, the hippocampus, the amygdala, and the thalamus (Kadar et al., 1992, 1995; McDonough and Shih, 1997; Aroniadou-Anderjaska et al., 2009). The severity of damage was found to correlate with the extent and duration of convulsions (McLeod, 1985; Carpentier et al., 2008; de Araujo Furtado et al., 2010). The prolonged inhibition of ChE and the accumulation of acetylcholine in the synapses, initiate a cascade of events leading to neuronal death (McDonough and Shih, 1997; Lallement et al., 1998).

The long term brain damage reported following sarin exposure is mostly irreversible and may even increase with time. The immediate and early histopathological and biochemical changes following the exposure leading to the brain damage have not been fully characterized. The assumption is that if attenuation of brain damage is to be achieved, effective treatment intervention should be introduced in a timely fashion during these early events.

One possible mechanism for neuronal death is apoptosis (Miller and Kaplan, 2001; Morrison et al., 2003). Apoptotic neurons have been previously described in brains of rats following OP insecticides exposure (Kaur et al., 2007; Gunay et al., 2010) and in brains from rat models for Alzheimer, Parkinson, Huntington disease, stroke and Amyotrophic lateral sclerosis (ALS). Fragmented DNA, cell body shrinkage, bulbs on the surface of the cells, chromatin degeneration and mitochondrial destruction were all found associated with the apoptotic process (Mattson, 2000). The proteins Bcl2, caspases, Fas and FasL, TNF and TNF receptors and a number of transcription factors such as c-fos, and Jun. were found to regulate the apoptotic process (Yuan and Yankner, 2000). The Bcl2 protein family includes the pro-apoptotic Bax and the anti-apoptotic Bcl2. These two key proteins control cell death (Youle and Strasser, 2008). In normal cells, Bax protein is localized mainly in the cytoplasm, but upon apoptotic signal, Bax changes its structural protein conformation, binds to the mitochondrial membrane (Gross et al., 1998; Pierrat et al., 2001), stimulates a voltage dependent anionic channel (Shi et al., 2003) and as a result, cytochrome C and other pro-apoptotic factors are released from the mitochondria, and caspases are activated (Weng et al., 2005). The mitochondrial damage initiate the

* Corresponding author at: Dept. of Pharmacology, Israel institute for Biological Research (IIBR), Ness Ziona 74100, Israel.
E-mail address: ettieg@iibr.gov.il (E. Grauer).

http://dx.doi.org/10.1016/j.taap.2016.09.008
0041-008X/

process that starts with the formation of free radicals that damage the DNA, lead to elevation in Jun.-N-terminal kinase (JNK) and other stress protein activity and ends in neuronal death (Fuchs et al., 1998). At the same time, an opposite process is triggered: as a result of the neuronal damage, NGF is secreted, Ras activity is elevated, raises Raf1 activity that activates MEK1 that activates anti-apoptotic ERK1/2 (Xia et al., 1995; Anderson and Tolkovsky, 1999).

A second type of neuronal cell death is necrosis. Cellular swelling, distension of various cellular organelles, clumping and random degradation of nuclear DNA, extensive plasma membrane endocytosis, and autophagy with subsequent inflammation (Hall et al., 1997; Ferri and Kroemer, 2001) mark necrosis. Necrotic cell death occurs generally in response to severe changes in physiological conditions, including hypoxia, ischemia, toxin exposure, extreme temperature changes and nutrient deprivation (Walker et al., 1988; Nicotera et al., 1999). Several neurodegenerative diseases, such as Alzheimer's, Huntington's, Parkinson, ALS and epilepsy (Price et al., 1998) also involve necrosis.

CNS damage is commonly comprised of various cell populations affected by the insult. We followed the changes of two of these populations: astrocytes (using GFAP, glial fibrillary acidic protein) and neurons (using NeuN, neuronal nuclei binding protein, and MAP2, microtubule-associated protein 2, for axons and dendrites). Astrocytes react to all forms of CNS insults through a process referred to as reactive astrocytosis. The changes in astrocytes range from cell hypertrophy with preservation of cellular domains and tissue structure, to long-lasting scar formation with rearrangement of tissue structure. Reactive astrocytes have the potential to play either primary or contributing roles in CNS disorders via loss of normal astrocytic functions or gain of abnormal effects (Hamby and Sofroniew, 2010; Sofroniew and Vinters, 2010; Sofroniew, 2013). Abnormal neurons will lose their antigenicity and therefore will not be identify by anti-NeuN antibody (Mullen et al., 1992; Collombet et al., 2006, 2008). MAP2 is one of the abundant microtubule-associated cytoskeletal protein in the neuronal cells. This protein is tightly connected to cytoskeletal elements that preserve and stabilize the normal cytoskeletal structure (Dhamodharan and Wadsworth, 1995; Maccioni and Cambiazo, 1995). Several reports showed that hyperactivity of the cholinergic system caused hyper-phosphorylation of MAP2 and damaged its ability to connect with other microtubule proteins, which led to dendritic abnormalities and to memory aberrations (Johnson and Jope, 1992; Woolf, 1999; Sanchez et al., 2000). Other works demonstrated that neurotoxicity as a result of ischemic brain injury, traumatic brain injury (Kitagawa et al., 1989; Chauhan et al., 1993) or OP exposure (Katalan et al., 2013) caused a decrease in MAP2 labeling in the affected brain areas.

The current study aimed to test the involvement of apoptotic process in nerve cell destruction and to characterize the histopathological damage induced by sarin 1LD50 at early time points following exposure. Better understanding of the biochemical and molecular mechanisms leading to neuronal damage at early stages following sarin insult should guide the specific and timely intervention in the attempts to minimize or even prevent the ensuring brain damage.

2. Methods

2.1. Animals

Male albino Sprague-Dawley rats weighing 280-300 g at the beginning of the experiment were purchased from Charles River (England). All procedures involving animals were in accordance with the Guide for the Care and Use of Laboratory Animals, National Academy Press, Washington, DC, 1996, and were approved by the Institutional Animal Care and Use Committee. Animals were housed three in cage, in a controlled environment with a temperature of 21 ± 2 °C and a 12-h light/dark cycle with lights on at 6 am. Food and water were available at lib.

2.2. Materials

Sarin (isopropyl methylphosphono-fluoridate) was supplied by the department of Organic Chemistry of IIBR, dissolved in propylene glycol and kept frozen. Fresh dilute solutions in saline were prepared for each experiment. Polyclonal rabbit anti-GFAP was purchased from Dako (Glostrup, Denmark). Monoclonal mouse anti-NeuN was purchased from Chemicon (MA, USA). Monoclonal mouse anti-MAP2, Monoclonal mouse anti-Bax and Monoclonal mouse anti-Bcl2 were purchased from Santa Cruz Biotechnology Inc. (CA, USA). Monoclonal mouse anti-pERK1/2, Monoclonal mouse anti-pJNK, Polyclonal rabbit anti-ERK1/2 and Polyclonal rabbit anti-JNK were purchased from Sigma (Rehovot, Israel). Secondary antibodies Goat anti-mouse-alexa fluor 488/594 and Goat anti-rabbit- alexa fluor 594 were purchased from Molecular Probes (OR, USA). Secondary HRP-conjugated antibodies Donkey anti-mouse-HRP and Donkey anti-rabbit-HRP were purchased from Jackon Immuno Research Laboratories Inc. (PA, USA). Mouse anti-neuronal class III β-tubulin (Tuj1) was purchased from Covance (CA, USA). DAPI, Cresyl violet, Trisma base, EDTA, EGTA, β-glycerophosphate, Sodium chloride, Benzanodine, Sodium ortho-vanadate, Chloroacetic acid, Sodium hydroxide, PMSF, DTT, Pepstatin A, Leupeptin, Aprotinine, Isoamyl alcohol, TriReagent, Agarose and BSA were all purchased from Sigma (Rehovot, Israel). Natural-buffered 4% PFA pH = 7.0 was purchased from Gadot (Rehovot, Israel) Fluoromount-G was purchased from Southern Biotechnology (AL, USA). Xylene based mounting medium was purchased from Lipshaw (PA, USA). Difco TM skimmed milk was purchased from BD (MD, USA). ECL and [^3H]ACh were purchased from Perkin elmer Inc. (MA, USA). Sentilation liquid Quicq Safe N was purchased from Zynsser analytic (Frankfurt, Germany). In-situ cell death detection kit-fluorescein was purchased from Roch (Mannehem, Germany).

2.3. Study design

Rats were exposed to Sarin (80 μg/kg, i.m., 1LD50) and euthanized by decapitation 2 h, 6 h, 24 h and 48 h following exposure. Brains were rapidly excised and processed for biochemical analysis (ChE inhibition, TSPO mRNA analysis and binding activity, Bax, Bcl2 mRNA and protein assessment and ERK1/2 and JNK activity estimation) and for histological evaluation (Nissle stain, Tunnel assay, GFAP, NeuN, MAP2).

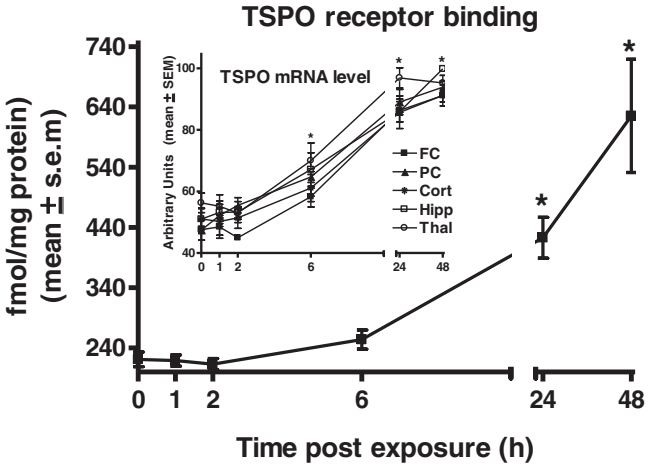

Fig. 1. Increase in TSPO receptor density and in mRNA level (insert) in brains of rats exposed to 1LD50 sarin (80 μg/kg, i.m.) and sacrificed 1 h, 2 h, 6 h, 24 h and 48 h post exposure. n = 5/time point, *$p < 0.0001$ vs. control.