Drug and Chemical Toxicology

ISSN: 0148-0545 (Print) 1525-6014 (Online) Journal homepage: http://www.tandfonline.com/loi/idct20

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To cite this article: Barry W. Wilson, John D. Henderson & Peter S. Spencer (1998) Clinical Effects of Low-Level Exposures to Chemical Warfare Agents in Mice and Chickens, Drug and Chemical Toxicology, 21:sup1, 183-190, DOI: 10.3109/01480549809007410

To link to this article: https://doi.org/10.3109/01480549809007410

Published online: 20 Oct 2008.

DRUG AND CHEMICAL TOXICOLOGY, 21(SUPPL. 1), 183-190 (1998)

CLINICAL EFFECTS OF LOW-LEVEL EXPOSURES TO CHEMICAL WARFARE AGENTS IN MICE AND CHICKENS

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ABSTRACT

Concerns that chemical warfare (CW) agents themselves or in combination with other chemicals may cause long-term damage to nerve and muscle are reviewed and discussed. Experiments on mice and hens underway with agent GA and pyridostigmine bromide (PB) and their effects (either separately or together) are presented.

INTRODUCTION

Much is known of the acute effects of anti-cholinergic chemical warfare (CW) agents and related less toxic organophosphate ester (OP) pesticides.[1] There has been less study of the possible effects of low level, repeated exposures or of the effects of mixtures of CW agents and other neuroactive chemicals such as carbamates like pyridostigmine bromide (PB).[2] The research project described here uses mice and chickens to study whether prior treatment with PB will affect the neurotoxicity of CW agent GB (Sarin; O-isopropyl, methylphosphonofluoridate).

BACKGROUND

CW agents such as GB, GA (Tabun; O-ethyl,N,N-dimethylphosphoroamidocyanidate), VX (O-ethyl,S-((N,N-diisopropyl-2-amino)ethyl)-methylphosphonothiolate) and GD (Soman;

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O-pinacolyl, methylphosphonofluoridate) have been manufactured, stockpiled, and tested on experimental animal and humans since the 1930's.[3] The recent release of GB from a bunker during the Gulf Conflict, the need for safe programs to destroy stockpiled agents and recent terrorist incidents in Japan have heightened the concern of policy makers and the public about the risks to society of these neurotoxic chemicals.[4]

Acute exposure to CW agents leads to muscarinic (neural) and nicotinic (muscle) receptor effects, often attributed to increased acetylcholine (ACh) and recently to direct actions of the agents themselves. These include the oft mentioned symptoms abbreviated as SLUD (salivation, lacrymation, urination and defecation), as well as muscle weakness, convulsions and death.[5]

Long-term Effects of Nerve Agents

Long-term effects of CW agents and related OPs studied on experimental mammals and birds and described in humans include a polyneuropathy (Organophosphate Induced Delayed Neuropathy, OPIDN), a shorter term myopathy (ACh Myopathy) a so-called Intermediate Syndrome, respiratory, facial and muscle weakness.[5,6,7,8] All have been demonstrated after administration of relatively high doses.

OPIDN

OPIDN has been studied since 1931, when it was recognized that a contaminant of bootleg liquor, tri-ortho cresyl phosphate (TOCP), caused a delayed axonal degeneration followed by myelin breakdown in chickens. An accidental spill of the OP mipafox that researchers focused attention on pesticide OPs, ultimately leading to screening them for neuropathic potential. A special carboxylesterase, neuropathy target esterase (NTE), was identified as being associated with OPIDN. Screening for delayed neuropathy usually involves hens, the appearance of ataxia, inhibition of brain NTE and retrograde axonal degenration of long nerve fibers in the central nervous system (CNS) and peripheral nervous system (PNS).[6]

Among CW agents, only GB has been shown to severely inhibit NTE and cause OPIDN in hens when given in single doses at levels requiring administration of protectants such as atropine and 2-PAM (Table 1, Table 2).[9,10]

Table 1. OPIDN in Hens After CW Agents

Agent	mg/kg	Survive	% NTE	OPIDN
Sarin (GB)	1.8	8/8	32	0/4
	3.6	8/8	18.5	4/4
	7.2	9/11	12.2	5/5
	14	3/6	---	3/3
Soman (GD)	2.2	12/19	45.4	0/8
Tabun (GA)	25	8/26	34	0/2
	50	2/6	---	0/2

Birds given physostigmine, atropine, 2-PAM protection.
(Adapted from Gordon et al.)[9]

Table 2. NTE Levels of Hen Brain after Acute Treatment with Agent VX

Treatment	2 day	4 day
Control	30.2 ± 1.4	27.9 ± 1.3
TOCP	0.58	2.46
	(n=1)	(n=2)
VX	34.8 ± 2.4	26.2 ± 3.1

Units: nmol phenyl valerate hydrolyzed/min/mg protein
Birds pretreated with atropine and 2-PAM; TOCP (400 mg/kg s.c.), VX (150 ug/kg s.c.)
n=3 except where indicated.
(After Wilson et al.)[10]

Research at UC Davis in which hens were injected with agent after atropine protection for 5 days each week for 90 days did not reveal inhibition of NTE, ataxia or histological damage in birds treated with tabun, VX, or paraoxon. Birds treated with DFP showed evidence of the polyneuropathy including ataxia and histopathological lesions to axons.[11] Recently several groups have reported inhibition of NTE, brain cell lesions, and ataxia with agent GB as well as with mipafox and soman in rodents (Table 3, Table 4).[12,13]

ACh Myopathy

Pathological changes separate and apart from OPIDN may occur at the neuromuscular junction and associated musculature following acute intoxication with OPs. In rodents, it was found that localized muscle fiber necrosis appeared within 24 hours and lasted for up

Table 3. NTE Levels in Mouse Tissues Exposed to Sarin or Mipafox

Treatment	Brain	Sp. Cord	Platelet	Mean
Control (n=8) (units)	681	259	4.04	
Sarin (n=6) (Percent)	40.7	52.5	44.6	45.9
Mipafox (n=6) (Percent)	34.5	43.6	39.6	39.2

Units: nmol phenyl valerate hydrolyzed/min/g tissue or mg protein
Sarin (5 mg/m^3, 20 min inhalation), mipafox (2.5 mg/kg s.c.)
Treated daily for 10d, sacrificed on day 14.

(Adapted from Husain et al.) [12]

Table 4. Axonal Degeneration of Spinal Cord of Mice After Sarin or Mipafox

Control	Sarin	Mipafox
0	1.33	2.67

Mean extent of degeneration in lateral columns, scale of 0-3.
Sarin (5 mg/m^3, 20 min inhalation), mipafox (2.5 mg/kg s.c.)
Treated daily for 10d, sacrificed on day 14.

(Adapted from Husain et al.)[12]

Table 5. Muscle Fiber Necrosis of Rat Muscle After DFP and Btx

Treatment	EDL	SOL
Saline	0	0
DFP	85.5	28.0
Btx	0	0
DFP + BtX	0	1.59

DFP injected 48 after Btx, sampled 24 h later.
1.5 mg/kg sc; 5 mice units, Necroses/1000 fibers

(After Sket et al., 1991)[17]

to several weeks after exposure to OPs.[7] Studies from several laboratories provided evidence that the damage was due to excessive ACh and over-stimulation of post-synaptic receptors.[14,15,16] Salpeter and her colleagues established that Ca^{2+} influx was part of the mechanism using in vitro muscle slices and Meshul demonstrated the the effect of Ca^{2+} in vivo.[15,16] For example, Table 5 from a study of Sket et al., 1991 shows that treatment with the ACh receptor blocker alpha-bungarotoxin (Btx) prevents identifiable necrotic regions in rat muscle fibers .[17] ACh Myopathy mostly has been studied in experimental animals. Findings with humans include the report of Wecker et al. of necrotic fibers at autopsy in intercostal muscles of a victim of OP poisoning.[18]

Intermediate Syndrome

In 1987, Senanayake and Karalliede reported a prolonged OP neuromuscle toxicity in humans after exposure to OP pesticides and coined the term "Intermediate Syndrome".[8] DeBleecker et al. noted both presynaptic and post-synaptic effects.[19]

Matsumoto and Tokyo

Agent GB has been used in Japan by terrorists in at least two incidents, a sobering prelude to what could occur in the 21st Century. A study of the victims of exposure to agent GB in Matsumoto and Tokyo provide evidence for long term muscle and nerve damage as well as other CNS and neurobehavior effects.[20] It is not clear what part of such long-term effects can be attributed to the ischemia that accompanies acute exposure to anti-cholinergic agents and which may involve chemically induced myopathies and neuropathies.

Pyridostigmine and Chemical Mixtures

Much research has been conducted to establish PB as a valid prophylactic drug to decrease the impact of CW agents.[2] Nevertheless, reports of adverse effects of PB in experimental animals and man, and adverse interactions between OPs and PB in experimental animals, have lead to speculations concerning the role played by such mixtures in long-term neurological symptoms described in Gulf veterans.[21, 22, 23, 24, 25,26]

Research is needed on several fronts to establish sensitive No-Adverse Effect Levels, to

document the effects of mixtures, and to devise biomarkers of long-term effects in addition to the inhibition of NTE and acetylcholinesterase.

Current Project

We have recently initiated a project using mice and chickens to find out whether agent GB will induce a sub-clinical peripheral neuropathy or a myopathy, and whether its intensity will be affected by PB. We will be examining brain, nerve and muscle for potential toxicity, identifying the sites that are particularly vulnerable to damage by the nerve agent. The purpose of the study is to provide a foundation to assess the risk of human populations that may have been in the past or may be in the future exposed to low levels of GB. Biochemistry is being performed on brain, spinal cord, peripheral nerve and muscle to study enzyme such as AChE and NTE by Wilson's team at UC Davis[10,27,28]. Morphometry of the neuromuscular systems will be done in Oregon by Spencer's group.[29,30] Brain, spinal cord, peripheral nerves and associated muscle will be examined by light and electron microscopy. Agents under study include GB, DFP and paraoxon. Task One is underway: it is a scoping trial of the agents to establish appropriate dose/response ranges. Task Two will be to determine threshold and relative dose/effect levels for both biochemical and morphometric tests. Tasks Three and Four will focus on pretreating animals with PB and then with either agent GB, DFP or paraoxon. We hope to find out how much and how often exposure to GB is needed to produce sub-clinical effects, which regions of the nervous system are most sensitive to GB and whether PB will modulate the toxicity of GB.

ACKNOWLEDGEMENTS

Presented at the 1998 Biomedical Defense Bioscience Review. Parts of this paper adapted from a Satellite Symposium of the 36th Society of Toxicology Annual Meeting (March 7-8, 1997, Cincinnati, Ohio), "Health Effect of Low-level Chemical Warfare Nerve Agent Exposure," sponsored by the Department of Veterans Affairs. This work is supported in part by the U.S. Army Medical Research and Materiel Command under Contract No. DAMD17-97-C-7057. The views, opinions and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation. In conducting research using animals, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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