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Comparison of the lethal effects of chemical warfare nerve agents across multiple ages

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HIGHLIGHTS

- Children may be more vulnerable than adults to chemical warfare nerve agents (CWNA).
- We estimated the 24 h LD_{50} for seven CWNA in rats at six times in development.
- Perinatal and adult rats were more susceptible than pubertal rats to the G-agents.
- Age-related differences were not observed in rats for the V-agents.

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ABSTRACT

Children may be inherently more vulnerable than adults to the lethal effects associated with chemical warfare nerve agent (CWNA) exposure because of their closer proximity to the ground, smaller body mass, higher respiratory rate, increased skin permeability and immature metabolic systems. Unfortunately, there have only been a handful of studies on the effects of CWNA in pediatric animal models, and more research is needed to confirm this hypothesis. Using a stagewise, adaptive dose design, we estimated the 24 h median lethal dose for subcutaneous exposure to seven CWNA in both male and female Sprague-Dawley rats at six different developmental times. Perinatal (postnatal day [PND] 7, 14 and 21) and adult (PND 70) rats were more susceptible than pubertal (PND 28 and 42) rats to the lethal effects associated with exposure to tabun, sarin, soman and cyclosarin. Age-related differences in susceptibility were not observed in rats exposed to VM, Russian VX or VX.

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1. Introduction

Chemical warfare nerve agents (CWNA) are historically divided into two categories that differ based on their physical properties and potential for toxicity: the G-series consisting of tabun (GA), sarin (GB), soman (GD) and cyclosarin (GF) and the V-series consisting of VM, Russian VX (VR) and VX. The G-series of CWNA are more volatile and pose an inhalation hazard, whereas the V-series are less volatile, more viscous and pose a dermal hazard (reviewed in Munro et al., 1994). Both categories of CWNA exert their toxicological effects by inhibiting acetylcholinesterase, the enzyme responsible for hydrolyzing the neurotransmitter acetylcholine in the central and peripheral nervous systems. The accumulation of acetylcholine within the synaptic cleft following extensive acetylcholinesterase inhibition prolongs the stimulation of muscarinic and nicotinic receptors on autonomic ganglion,

Abbreviations: ANOVA, analysis of variance; BARDA, Biomedical Advanced Research and Development Authority; CWNA, chemical warfare nerve agent(s); CI, confidence interval; GF, cyclosarin; ECBC, Edgewood Chemical Biological Center; LD_{50}, median lethal dose; PND, postnatal day; VR, Russian VX; GB, sarin; GD, soman; sc, subcutaneous(ly); GA, tabun; USAMRICD, US Army Medical Research Institute of Chemical Defense.

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end-organs, myocytes and postsynaptic neurons, which leads to an acute cholinergic crisis characterized by autonomic and cardiac dysfunction, involuntary movements, miosis, muscle fasciculations, respiratory failure, salivation, seizures and ultimately death if left untreated (reviewed in Pereira et al., 2014). The tragic consequences of CWNA exposure were observed on 21 August 2013 when GB was released amongst a civilian population residing in the outskirts of Damascus, Syria (Rosman et al., 2014; United Nations General Assembly, 2013).

Children are predicted to be more vulnerable to CWNA exposure than adults because of their closer proximity to the ground, greater surface area to body mass ratio, faster respiration rate, increased skin permeability, immature detoxification systems and higher risk for seizures following a neurotoxic insult (reviewed in Rotenberg and Newmark, 2003). However, the results from the few studies that have examined the effects of CWNA exposure in pediatric animal models are conflicting. Postnatal day (PND) 5 rats subcutaneously (sc) exposed to GD have lower median lethal dose (LD_{50}) values than PND 30 rats (Sterri et al., 1985). Similarly, PND 21 rats sc exposed to GD have lower LD_{50} values than young-adult rats (PND 35–40 based on the reported body weight range), as well as shorter onsets to behavioral seizures (Miller et al., 2015). In contrast, PND 30 rats intramuscularly exposed to GD have higher LD_{50} values than PND 60, 120 and 240 rats (Shih et al., 1990). PND 30 rats also exhibit less weight loss and more rapid growth recovery in the two weeks following exposure than do the older age groups. To add to the confusion, the lethal effects associated with sc exposure to GD are not age-dependent in the guinea pig model (Fawcett et al., 2009). However, neonatal (PND 5–10) and prepubertal (PND 35–45) guinea pigs sc exposed to GB or VX have higher LD_{50} values than adults (PND 120–150). Thus, it is unclear whether susceptibility to the lethal effects of CWNA exposure decreases or increases with the age of the animal model.

The goal of this study was to provide a more comprehensive evaluation of the lethal potencies of CWNA during the development of a rat. Using a stagewise, adaptive dose design, we estimated the 24 h LD_{50} for sc exposure to seven CWNA (GA, GB, GD, GF, VM, VR and VX) in male and female rats at six different times (PND 7, 14, 21, 28, 42 and 70). These times were selected to span from the brain growth spurt period (Dobbing and Sands, 1971) into puberty (Spear, 2000) and early adulthood, and a high-throughput, parental route of exposure was chosen as opposed to a more operationally relevant route (e.g. inhalation or dermal) to minimize the variability in absorption of the CWNA. The subcutaneous route was chosen as opposed to the intravenous or intramuscular routes because of its relative ease with perinatal rats. Identical methodology, something lacking in earlier studies, was used for each CWNA to deliver a reliable account of their age- and sex-related effects in the rat model.

2. Methods

2.1. Animals

Male and female Sprague-Dawley rats (CD IGS) were purchased from Charles River Laboratories International, Inc. (Kingston, NY) and age-matched into six groups (PND 7, 14, 21, 28, 42 and 70). Lactating dams with litters (five males and five females per litter) were purchased for the three youngest age groups. PND 7 and 14 rats remained with their dams throughout the experiment, while PND 21 rats were weaned approximately 1 h before exposures. The pre-exposure weights of each age group and sex across all CWNA studies are listed in Table 1. Rats were housed in temperature- and humidity-controlled colony rooms ($21 \pm 2\,^{\circ}C$ and $60 \pm 20\%$, respectively) under a 12:12 h light:dark cycle (lights on at 06:00 hours). Food was withheld for 1 h post-exposure to minimize the risk of choking. Otherwise, food and water were available *ad libitum*. The experimental protocol was approved by the Animal Care and Use Committees at the US Army Medical Research Institute of Chemical Defense (USAMRICD) and Edgewood Chemical Biological Center (ECBC), and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

2.2. Agent exposures

GA (ethyl dimethylamidocyanophosphate), GB (isopropyl methylphosphonofluoridate), GD (pinacolyl methylphosphonofluoridate), GF (cyclohexyl methylphosphonofluoridate), VM (*O*-ethyl *S*-(2-diethylaminoethyl) methylphosphonothioate), VR (*O*-isobutyl *S*-(2-diethylaminoethyl) methylthiophosphonate) and VX (ethyl-*S*-dimethylaminoethyl methylphosphonothioate) were obtained from ECBC's chemical agent standard analytical reagent material stock (approximate concentration: 2 mg/ml for G-agents and 1 mg/ml for V-agents; solvent: physiological saline) and diluted to the appropriate concentration (3.4–428.5 μg/ml) with physiological saline on the morning of exposures. Exposures were conducted between 09:00 and 12:00 hours, and a split-litter design was used such that individual offspring of the same sex in

Table 1
Body weight (mean ± standard deviation) for all male and female rats studied at various times in development. The body surface area for each animal was calculated using the equation of Spiers and Candas (1984), which takes into account the various stages of development. This value was then divided by the animal's body weight to give a body surface area to body weight ratio. A two-way analysis of variance (ANOVA) with age group and sex as factors was conducted to determine differences in these ratios. There was a significant interaction between the two factors ($F(5,2049) = 54.2$, $p < .001$); thus, a one-way ANOVA followed by a Bonferroni *post hoc* test was conducted for each sex to determine differences between age groups. The differences are notated as follows:

	Male				Female			
Group	Weight (g)	Surface area (cm^2)	Ratio (cm^2/g)	N	Weight (g)	Surface area (cm^2)	Ratio (cm^2/g)	N
PND 7	18 ± 2.7	58 ± 6.3	3.21 ± 0.12[a,b,c,d,e]	170	17 ± 2.5	56 ± 6.0	3.26 ± 0.13[a,b,c,d,e]	176
PND 14	32 ± 2.7	89 ± 5.4	2.75 ± 0.06[b,c,d,e]	178	31 ± 2.7	87 ± 5.5	2.78 ± 0.06[b,c,d,e]	174
PND 21	53 ± 7.4	128 ± 12.9	2.42 ± 0.08[c,d,e]	161	50 ± 5.4	122 ± 9.8	2.45 ± 0.07[d,e]	166
PND 28	92 ± 9.2	192 ± 14.0	2.09 ± 0.05[d,e]	172	84 ± 7.8	180 ± 12.2	2.14 ± 0.05[d,e]	148
PND 42	226 ± 19.6	371 ± 23.7	1.65 ± 0.04	185	167 ± 13.4	297 ± 17.4	1.78 ± 0.04	190
PND 70	329 ± 21.8	489 ± 23.8	1.49 ± 0.02	185	225 ± 16.9	371 ± 20.3	1.65 ± 0.03	156

[a] Significantly different from PND 14, $p < .05$.
[b] Significantly different from PND 21, $p < .05$.
[c] Significantly different from PND 28, $p < .05$.
[d] Significantly different from PND 42, $p < .05$.
[e] Significantly different from PND 70, $p < .05$.