Sarin Nerve Gas - Or How I Learned to Stop Worrying and Love Pon-1

Beth Simpson

Sarin (Figure 1) is an odourless, colourless, volatile liquid that acts as a highly toxic nerve agent capable of killing within minutes. Sarin is a member of a class of chemicals known as organophosphates, which also includes other nerve agents, such as tabun, soman and VX (Figure 1 c-3), and pesticides, such as paraoxon. It was first synthesized by German chemists in 1937 as a pesticide. Its potential as a chemical weapon was recognized, and Germany stockpiled, but did not use, sarin during World War II. Sarin has since been used in military combat, specifically in the Iran-Iraq conflict in the 1980s. In addition, sarin has been used by a terrorist organization, the cult Aum Shinrikyo in Japan, in a residential area of the city of Matsumoto in 1994 and in the Tokyo subway system in 1995. These attacks resulted in 7 deaths and 58 hospitalizations, and 12 deaths and over 6000 individuals seeking medical attention, respectively.

The threat of terrorist attack using sarin is very real. The chemicals needed to synthesize sarin - dimethyl methylphosphonate, phosphorus trichloride, sodium fluoride and alcohol - are readily available. Reporters from both Scientific American (Musser, 2001) and the British Broadcasting Corporation (BBC) (Stickler, 2003) were able to obtain all the chemicals necessary for the production of sarin through chemical supply companies. The recipe for sarin is readily available, both on the Internet and in books such as "Silent Death", which can be purchased through online book retailers such as Amazon.com, Barnes & Nobles and Direct Textbook (Direct Textbook, 2003). In addition, the delivery of sarin is relatively easy - in Matsumoto, sarin was released by placing it on a heated surface to rapidly vaporize it and then blowing it into the air with a fan; in Tokyo,

bags filled with sarin were punctured with umbrella tips as the terrorists exited subway trains (Nerve Agent, 2003).

In addition to exposure during military combat and terrorist attacks, the possibility of accidental exposure to sarin during its disposal and the secondary exposure of emergency response personnel (before it is recognized that sarin has been used) are very real dangers. For example, at the end of the 1991 Gulf War, munitions containing approximately 8.5 metric tons of sarin and a related nerve agent, cyclosarin (Figure 1b), were destroyed by US military personnel at Khamisiyah, Iraq (McCauley et al., 2001). At the time, the US Department of Defense did not know that the munitions contained chemical weapons and therefore no precautions to protect personnel were
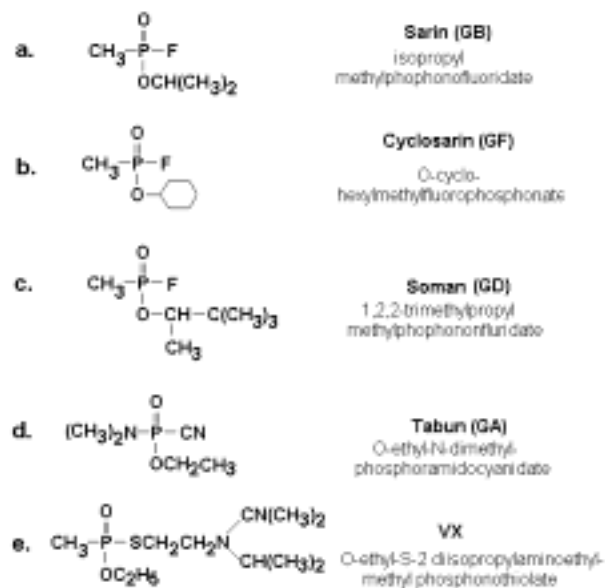
Figure 1. The structure of various nerve agents

Table 1: Symptoms of Sarin Exposure
Muscarinic Symptoms:·
marked miosis (pinpoint pupils), eye pain, blurred vision·
rhinorrhea (runny nose)·
dyspnea (shortness of breath)·
increased bronchial secretion·
nausea, vomiting, abdominal cramps, involuntary defecation, involuntary urination·
increased sweating, salivation, lacrimation (shedding of tears)·
bradycardia (slowed heart rate)
Nicotinic Symptoms:·
fatigue·
muscle weakness, twitching and fasciculations (small, local muscle contractions)·
generalized weakness or flaccid paralysis (including respiratory muscles)·
transitory hypertension (high blood pressure), followed by hypotension (low blood pressure)
Central Nervous System Effects:·
depression of respiratory and circulatory centers (with dyspnea)·
convulsions·
loss of consciousness·
coma

(adapted from IOM 2000)

Table 1. Symptoms of sarin exposure.

taken. The US Army is currently destroying some of its own stockpile of sarin and other chemical weapons (as required under the Chemical Weapons Convention (OPCW, 1993)) at a weapons incinerator in Anniston, Alabama (Army begins, 2003). Approximately 35,000 people who live within nine miles of this incinerator have been issued gas masks and shelter kits, and local schools are being pressurized to keep fumes out, in the event of accidental chemical weapons release from the facility (Army begins, 2003).

There is a possibility that sarin exposure may occur through military, terrorist and occupational events. It is essential that public health professionals and healthcare workers are aware of the signs and symptoms of sarin exposure and of the available treatments for sarin intoxication. Furthermore, there is a pressing need for research into the acute and long-

term effects of sarin exposure and into new avenues for effective treatments against this deadly chemical weapon. This paper reviews the mechanisms of sarin toxicity, the potential long-term health effects of low-level sarin exposure, the currently available treatments for sarin intoxication and the future directions of research into new treatments.

Mechanisms of Sarin Toxicity

Exposure to sarin, which can be absorbed across the respiratory tract, eyes, mucous membranes and skin, can be fatal within minutes. A dose of 50-100 mg/min/,m3 by inhalation, or 100-500 mg across the skin, is lethal to 50% of those exposed (IOM, 2000).

Sarin works by irreversibly binding to the enzyme acetylcholinesterase (AChE), which results in the inactivation of this enzyme. AChE breaks down the neurotransmitter acetylcholine (ACh); thus, when AChE is inactivated by sarin, ACh builds up in

Specifically, sarin binds to AChE by phosphorylating the hydroxyl group of a serine residue in the active site of the enzyme, blocking the enzyme from interacting with its normal substrate, ACh. If sarin is not removed from AChE (by treatment with an oxime) within a few hours of exposure, AChE will undergo a dealkylation process known as "aging" in which the phosphorylated AChE becomes resistant to hydrolysis and is considered irreversibly bound to sarin and thus, irreversibly inhibited.

In addition to its effects on AChE, sarin has also recently been shown to interact directly with muscarinic ACh receptors (Chebabo, Santos & Alburquerque, 1999) and to inhibit the release of ã-aminobutyric acid (GABA), an inhibitory neurotransmitter. Reductions in the level of GABA are believed to be related to the convulsions caused by sarin.

Long-Term Health Effects of Sarin Exposure

While the effects of acute exposure to high doses of sarin are clear, the long-term effects of exposure to sarin are not as well known. There is significant interest in this area as it has been hypothesized that the chronic illness experience by some troops who served during the first Gulf War (sometimes referred to as "Gulf War Syndrome") may be due, at least in part, to low-level

exposure to sarin. In addition, the long-term health effects of sarin have implications for survivors of the terrorist attacks in Japan.

Long-term health effects of sarin exposure that have been reported include a decrease in memory in emergency rescue personnel three years after the Tokyo subway attack (Nishiwaki , Maekawa, Ogawa, Asukai, Minami & Omae, 2001); physical (eye symptoms, fatigue, muscle stiffness and headache) and psychological symptoms in survivors of the Tokyo subway attack 5 years later (Kawana, 2001); fatigue, headache and visual disturbances (blurred vision, eye pain) 1 to 3 years after the Matsumoto attack (Nakajima *et al.*, 1999) and changes to electroencephalogram (EEG) readings, indicating subtle central nervous system effects, in survivors of the Tokyo attack after six to eight months (Murata *et al.,* 1997).

In addition, animal studies have found that subclinical doses of sarin impair T cell responses and decrease corticosterone levels (Kalra *et al.*, 2002) and result in alterations in muscarinic receptor sites in the brain that may be associated with memory loss and cognitive dysfunction (Henderson *et al.,* 2002) in Fisher 344 rats (a standard animal model).

Clearly, more research into the long-term health effects of sarin-exposure is needed. This is complicated by a number of factors, including a lack of information about the dose of sarin experienced by survivors of the terrorist attacks in Japan and difficulty in differentiating between the effects of sarin itself and the effects of the stress of surviving a terrorist attack (e.g., post-traumatic stress disorder). Similarly, studies of Gulf War veterans who may have been exposed to sarin by the destruction of chemical weapons-filled munitions at Khamisiyah, Iraq are complicated by lack of information about which individuals were actually exposed and the dose to which they were exposed, and the effects of co-exposure to other chemical weapons that were present (e.g., cyclosarin) and to the pre-treatment carbamate drug, pyridostigmine bromide (McCauley *et al*, 2001).

Current Treatments for Sarin Intoxication
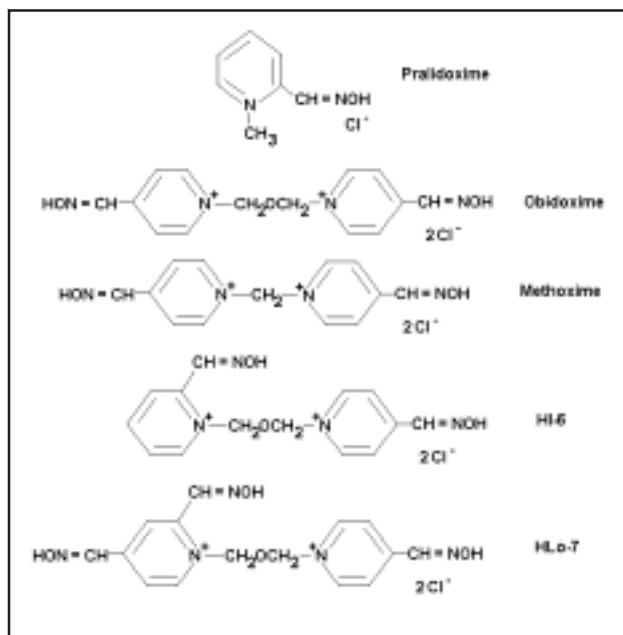
Figure 2 The structure of oximes

The current treatment for sarin exposure includes atropine, oximes and anticonvulsants (Lee, 2003; Taysee *et al*., 2003).

Atropine is used to treat sarin toxicity by antagonizing the effect of ACh at muscarinic ACh receptors, independent of AChE (Krejcova, Kassa & Vacek, 2002); thus, atropine results in a reduction of the muscarinic symptoms of sarin (Box 1), such as miosis, bronchial secretion, nausea, vomiting, abdominal cramps, involuntary defecation and urination, increased sweating, salivation and lacrimation and bradycardia, by blocking the overstimulation of ACh receptors. Since atropine cannot reactivate AChE, sarin toxicity may reoccur once the effects of atropine wear off if tissue concentrations of sarin are still high. Atropine is given until the muscarinic symptoms of sarin have been reversed and should be monitored by a trained medical professional to avoid atropine poisoning.

In addition to atropine, oximes (Figure 2) are considered standard treatment for sarin and other organophosphate toxicity. Oximes reactivate organophosphate-inhibited AChE by dephosphorylating the active site of the enzyme (Kassa, 2002). Several oximes are available with some (such as HI-6 and HLö-7) being more effective against nerve agents and others (such as pralidoxime and obidoxime) being more effective against
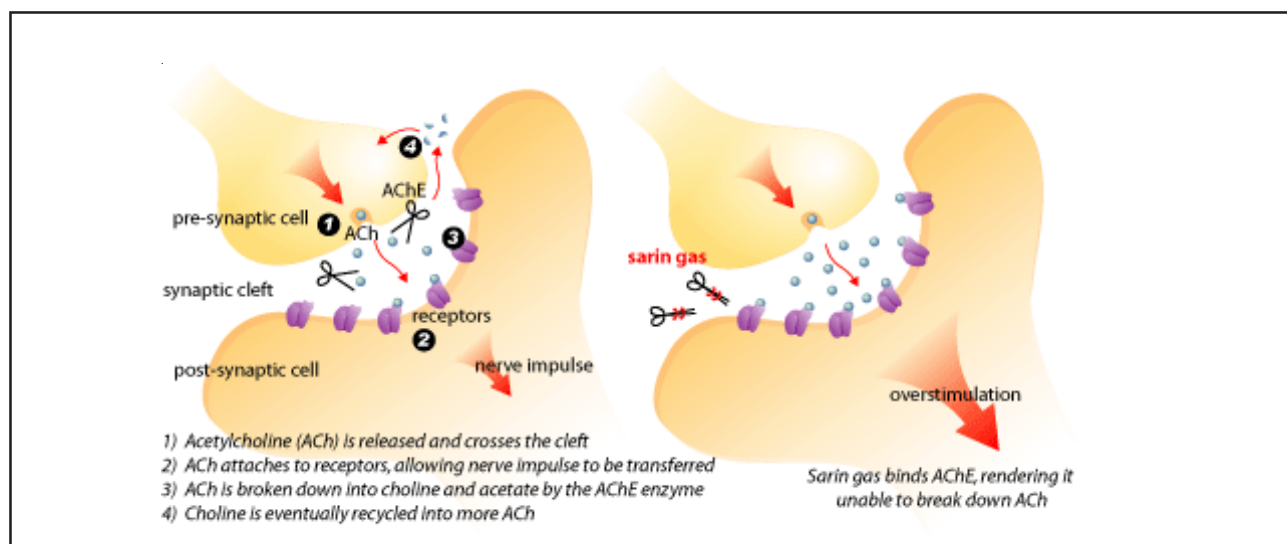
Figure 3. Mechanism of sarin gas toxicity.

organophosphate insecticides (Kassa, 2002). It should be noted that high levels of oximes can cause toxicity. Death by oxime toxicity is likely due to paralysis of respiratory muscles (Kassa, 2002), much like death due to sarin.

The final component of the current treatment for sarin is the use of an anticonvulsant, such as diazepam, to treat seizures associated with sarin toxicity. While diazepam is currently the anticonvulsant recommended for treating sarin-induced convulsions, a recent study in guinea pigs suggests that avizafone may competitive inhibitor to sarin by protecting AChE from the irreversible binding by sarin. Since carbamates exert a similar action to sarin, the side effects of these drugs are similar to the effects of sarin, including both nicotinic (muscle cramps, fasciculations and weakness) and muscarinic (nausea, vomiting, diarrhea, abdominal cramps, increase salivation, bronchial secretions, sweating and miosis) symptoms (IOM, 2000). Such effects can be transiently incapacitating to the patient.

Future Avenues of Research

Presently, pre- and post-exposure treatments for sarin toxicity have significant shortcomings. In addition, due to the rapid effects of sarin, it is often difficult to administer atropine, oximes and anticonvulsants in sufficient time to counteract the effects of sarin. While military personnel can be given autoinjectors containing atropine, oximes and anticonvulsants, it can be difficult for an individual to diagnose sarin exposure (as sarin

is colourless and odourless) and there is a risk of self-injecting accidentally or under the mistaken belief that one was exposed to sarin, creating a risk of atropine poisoning and oxime toxicity. While carbamates have the advantage of being a pre-treatment, and thus overcome the problems with diagnosing sarin exposure, they have significant negative effects that may be transiently incapacitating. This is of special concern to active military combatants and rescue personnel who are needed to carry out their duties in an emergency. Fortunately, there are some exploited as pre-treatments for sarin exposure.

While it is unlikely that naturally occurring levels of Gln_{192} PON-1 would be able to protect an individual against very high doses of sarin, due to sarin's high level of toxicity and rapidity of action, it is possible that the Gln_{192} isoform of PON-1 may help prevent effects of low-levels of sarin exposure by rapidly hydrolyzing the sarin that is encountered. In a study of 25 Gulf War veterans, Haley et al (1999) found that veterans ill with Gulf War Syndrome were more likely than controls to possess the Arg_{192} allele and to exhibit lower PON activity. While this small study needs to be confirmed with a larger population, it does lend support to the possibility that the Arg_{192} may represent a risk factor for health effects of low-level exposure to sarin (or, conversely, that the Gln_{192} genotype is protective of low-level sarin exposure).

The current research being conducted by the US Military to improve the binding affinity of PON-1 for

sarin, as well as the turnover rate (Broomfield & Kirby, 2001), looks to be a promising avenue of research that may well provide an effective and safe alternative to carbamates as a protection against sarin.

Conclusions

Exposure in Japan and Iraq demonstrate the reality of terrorism with sarin and other chemical weapons. The ease with which one can acquire sarin's ingredients and its method of synthesis, combined with the highly toxic and hard to detect nature of this deadly compound make it appealing to terrorists. The best line of defense against sarin starts with knowledge. A solid understanding of the mechanisms by which sarin induces toxicity will allow the development of more effective and less toxic treatments for sarin exposure. Training health care workers about the signs and symptoms of sarin intoxication will allow rapid diagnosis and treatment. Further research into the long-term health effects of low-level sarin exposure will help to reduce the uncertainty and anxiety experienced by survivors of sarin attack.

References

1. Musser, G. (2001). Better killing through chemistry. *Scientific American [On-line]*. Retrieved October 21, 2003, from www.sciam.com

2. Stickler, A. (2003). How I Obtained Sarin Ingredients. Retrieved October 21, 2003, from http://news.bbc.co.uk/1/hi/uk/2949356.stm

3. Direct Textbook (2003). Retrieved October 21, 2003, from www.directtextbook.com

4. Nerve Agent: GB (Sarin) (2003). Retrieved October 21, 2003, from www.cbwinfo.com/Chemical/Nerve/GB.shtml

5. McCauley, L.A., Rischitellie, G., Lambert, W.E., Lasarev, M., Sticker, D.L., & Spencer, P.S. Symptoms of Gulf War veterans possibly exposed to organophosphate chemical warefare agents at Khamisiyah, Iraq. *International Journal of Occupational and Environmental Health* **7**, 79-89. (2001).

6. Organization for the Prohibition of Chemical Weapons (2002). Chemical Weapons Convention. Retrieved October 21, 2003, from www.opcw.org

7. Army begins chemical weapons burn (2003). Retrieved October 21, 2003 from http://edition.cnn.com/2003/US/South/08/09/anniston.chemical.burn

8. Institute of Medicine (U.S.). (2000). *Gulf War and Health: Depleted Uranium, Pyridostigmine Bromide, Sarin, Vaccines. (Vol. 1)* Washington, DC: National Academy Press.

9. Chebabo, S.R., Santos, M.D., & Albuquerque, E.X. The organophosphate sarin, at low concentrations, inhibits the evoked release of GABA in rat hippocampal slices. *Neurotoxicology* **20**, 871- 882 (1999).

10. Nishiwaki, Y., Maekawa, K., Ogawa, Y., Asukai, N., inami, M.O., & Omae, K. Effects of sarin on the nervous system in rescue team staff members and police officers 3 years after the Tokyo subway sarin attack. *Environmental Health Perspective* **109**, 1169-1173 (2001).

11. Kawana, N. Psycho-physiological effects of the terrorist sarin attack on the Tokyo subway system. *Military Medicine* **166**, 23-26 (2001).

12. Nakajima, T., Ohta, S., Fukushima, Y., & Yanagisawa, N. Sequelae of sarin toxicity at one and three years after exposure in Matsumoto, Japan. *Journal of Epidemiology* **9**, 337-343 (1999).

13. Murata, K., Araki, S., Yokoyama, K., Okumura, T., Ishimatsu, S., Takasu, N., & White, R.F. Asymptomatic sequelae to acute sarin poisoning in the central and autnomic nervous system 6 months after the Tokyo subway attack. *Journal of Neurology* **244**, 601-606 (1997).

14. Kalra, R., Singh, S.P., Razani-Boroujerdi, S., Langley, R.J., Blackwell, W.B., Henderson, R.F., & Sopori, M.L. Subclinical doses of the nerve gas sarin impair T cell responses through the autonomic nervous system. *Toxicol.Appl.Pharmacol*. **184**, 82-87 (2002).

15. Henderson, R.F., Barr, E.B., Blackwell, W.B., Clark, C.R., Conn, C.A., Kalra, R., March, T.H., Sopori, M. L., Tesfaigzi, Y., Menache, M.G., & Mash, D.C. Response of rats to low levels of sarin. *Toxicol.Appl.Pharmacol*. **184**, 67-76 (2002).

16. Lee, E.C. Clinical manifestations of sarin nerve gas exposure. *JAMA* **290**, 659-662 (2003).

17. Taysse, L., Calvet, J.H., Buee, J., Christin, D., Delamanche, S., & Breton, P. Comparative efficacy of diazepam and avizafone against sarin-induced neuropathology and respiratory failure in guinea pigs: influence of atropine dose. *Toxicology* **188**, 197-209 (2003).

18. Krejcova, G., Kassa, J., & Vachek, J. Effect of atropine and the oxime HI-6 on low-level sarin-induced alteration of performance of rats in a T-maze. *Acta Medica.(Hradec.Kralove)* **45**, 107-110 (2002).

19. Kassa, J. Review of oximes in the antidotal treatment of poisoning by organophosphorus nerve agents. *J.Toxicol.Clin.Toxicol.* **40**, 803-816 (2002).

20. McRee, D. Protecting against cocaine, heroin, and sarin gas. *Chem.Biol.* **10**, 295-297 (2003).

21. Redinbo, M.R., Bencharit, S., & Potter, P.M. Human carboxylesterase 1: from drug metabolism to drug discovery. *Biochem.Soc.Trans.* **31**, 620-624.(2003).

22. Maxwell, D.M. and Brecht, K.M. Carboxylesterase: specificity and spontaneous reactivation of an endogenous scavenger for organophosphorus compounds. *J. Appl. Toxicol.* **21** Suppl 1:S103-7 (2001)

23. Broomfield, C.A. and Kirby S.D. Progress on the road to new nerve agent treatments. *J Appl Toxicol.* **21,** Suppl 1:S43-6. (2001).

24. Josse, D., Lockridge, O., Xie, W., Bartels, C.F., Schopfer, L.M., & Masson, P. The active site of human paraoxonase (PON1). *J.Appl.Toxicol.* **21** Suppl 1, S7-11 (2001).

25. Yamada, Y., Takatori, T., Nagao, M., Iwase, H., Kuroda, N., Yanagida, J., & Shinozuka, T. Expression of paraoxonase isoform did not confer protection from acute sarin poisoning in the Tokyo subway terrorist attack. *Int.J.Legal Med*. **115**, 82-84.(2001).

26. Clendenning, J.B., Humbert, R., Green, E.D., Wood, C., Traver, D., & Furlong, C.E. Structural organization of the human PON1 gene. *Genomics* **35**, 586-589 (1996).

27. Sierksma, A., van der Gaag, M.S., van Tol, A., James, R.W., & Hendriks, H.F. Kinetics of HDL cholesterol and paraoxonase activity in moderate alcohol consumers. *Alcohol Clin. Exp. Res.* **26**, 1430-1435 (2002).

28. Haley, R.W., Billecke, S., and La Du, B.N. Association of PON1 type Q (type A) aryl-esterase activity with neurologic symptom complexes in Gulf War veterans. *Toxicol.Appl.Pharmacol* **157**, 227-233 (1999).