J. Toxicol. Pub. Health
Vol. 16, No. 3, pp. 195-200, 2000

Protective Effect of Combinational Antidotes Composed of Physostigmine and Procyclidine Against Nerve-agent Poisoning

Yun-Bae Kim*, Ki-Cheol Cheon, Gyeung-Haeng Hur, Taek-San Phi, Jee-Cheon Kim and Deasik Hong

Biomedical Assessment Laboratory (GSDC-2-4), Agency for Defense Development, Yuseong P.O. Box 35-1, Taejon 305-600, Korea
(Received June 20, 2000)
(Accepted August 28, 2000)

ABSTRACT *: Antidotal efficacy of physostigmine plus procyclidine, the combinational prophylactics for organophosphate poisoning, was evaluated in rats and guinea pigs. To assess the dose-response relationship in rats, various doses (0.3~6.0 mg/kg) of procyclidine in combination with a fixed dose (0.1 mg/kg) of physostigmine were pretreated subcutaneously 30 min prior to subcutaneous exposure to nerve-agents. Physostigmine alone exerted protection ratios of 2.44, 1.20, 1.50, 1.50 and 2.20 folds for tabun, sarin, soman, cyclosarin and V-agent, respectively. Interestingly, coadmnistration of procyclidine with physostigmine exhibited remarkable synergistic effects in a dose-dependent manner, leading to 4.00~8.00 folds for tabun, 2.15~8.50 folds for sarin, 1.92~5.07 folds for soman, 2.15~2.90 folds for cyclosarin, and 2.71~10.50 folds for V-agent. On the contrary, a low effect (1.65 fold) was achieved with the traditional antidotes atropine (17.4 mg/kg) plus 2-pralidoxime (30 mg/kg) treated immediately after soman poisoning. Noteworthy, the combinational prophylactics markedly potentiated the effect of atropine plus 2-pralidoxime to 6.13 and 12.27 folds with 1.0 and 3.0 mg/kg of procyclidine, respectively, against soman poisoning. In guinea pigs, the physostigmine plus procyclidine prophylactics exerted protective effects of 3.00~4.70 folds against soman intoxcation, which were much higher at low doses (0.3~1.0 mg/kg) of procyclidine than those in rats. Taken together, it is proposed that the combinational prophylactics composed of physostigmine and procyclidine could be a promising antidote regimen for the poisoning with organophosphates possessing diverse properties.*

Key Words *: Physostigmine, Procyclidine, Nerve-agent poisoning*

I. INTRODUCTION

Organophosphates have been used worldwide as insecticides and are still one of the most-threatening warfare agents (Somani, 1992). Recently, organophosphates also emerged as a major threat of terrorism, since the Tokyo subway system had been subjected to attack with sarin (isopropylmethylphosphonofluoridate) gas that left more than 20,000 passengers poisoned and 12 persons dead (Nagao *et al.*, 1997; Suzuki *et al.*, 1995).

Organophosphates inhibit neural and blood cholinesterases, and thereby produce muscarinic and nicotinic signs according to the excessive accumulation of acetylcholine in cholinergic synapses and nerve terminals (Dunn and Sidell, 1989; Somani, 1992). In addition to cholinergic toxicities which may cause acute death, organophosphate poisoning induces centrally-originated convulsions, resulting in brain and cardiac injuries (Kim *et al.*, 1999; McDonough and Shih, 1993; Shih *et al.*, 1991; Tryphonas and Clement, 1995; Tryphonas *et al.*, 1996). It has been demonstrated that prolonged seizures longer than 20 min, following soman (pinacolylmethylphosphonofluoridate) poisoning, led to irreversible neuronal injuries (Shih *et al.*, 1991).

Atropine and 2-pralidoxime or obidoxime have been used for several decades as the standard treatment of organophosphate poisoning for their great synergistic antidotal effect on a broad spectrum of agricultural insecticides and warfare nerve-agents (Dunn and Sidell, 1989; Somani, 1992). Unfortunately, however,

*To whom correspondence should be addressed

the traditional antidotes did not exert synergistic protection against the lethality and brain injuries induced by soman or diisopropylfluorophosphate (Berry and Davies, 1970; Kim *et al.*, 1998) which undergo rapid dealkylation (*i.e.*, aging) after phosphorylation of acetylcholinesterase (Berman and Decker, 1986; Talbot *et al.*, 1988), since the oximes could not reactivate the aged enzymes.

To overcome the limited efficacy of atropine and 2-pralidoxime, tablets of pyridostigmine, a quaternary carbamate, have been used in armed forces as a prophylactic of nerve-agent poisoning (Dunn and Sidell, 1989; Keeler *et al.*, 1991), since the pretreatment with carbamates greatly improve the efficacy of antidotes in reducing the lethality induced by diverse organophosphates including soman and diisopropylfluorophosphate (Berry and Davies, 1970; Dirnhuber *et al.*, 1979; Gordon *et al.*, 1978). Also, an anticonvulsant diazepam, as an additional posttreatment injection, has been used in emergency situations (Clement and Broxup, 1993; Dunn and Sidell, 1989). However, diazepam, a γ-aminobutyric acid antagonist, was found to exhibit an insufficient neuroprotective activity (Philippens *et al.*, 1992) and to rather facilitate the respiratory suppression by soman poisoning which may lead to acute death (McDonough and Shih, 1993; Shih, 1990). Such results led the investigators to study on more effective and safe neuroprotective anticonvulsants (Lallement *et al.*, 1998; McDonough and Shih, 1993; McDonough *et al.*, 2000; Shih *et al.*, 1991).

In our recent studies, it was found that a novel combinational regimen composed of physostigmine and procyclidine exerted high antidotal, anticonvulsant and neuroprotective efficacies against diisopropylfluorophosphate poisoning in mice and rats (Kim *et al.*, 1998, 2000). Centrally-active physostigmine, a tertiary carbamate, was found to be more effective for the successful protection and the rapid recovery without severe physical incapacitation after survival than centrally-inactive pyridostigmine (Harris *et al.*, 1984; Kim *et al.*, 1998). In addition, procyclidine, possessing antimuscarinic (Waelbroeck *et al.*, 1990), antinicotinic (Gao *et al.*, 1998) and *N*-methyl-D-aspartate-antagonistic (McDonough and Shih, 1995) activities, rapidly eliminated the seizures leading to neuronal injuries (Kim *et al.*, 1997; McDonough *et al.*, 2000; Shih *et al.*, 1997), as inferred from that organophosphate-induced seizures were triggered by acetylcholine and promoted by excitatory amino acids accumulated following organophosphate poisoning (Lallement *et al.*, 1991; Mattson, 1989; McDonough and Shih, 1993; Shih *et al.*, 1991).

In the present study, we presents the protective effect of physostigmine plus procyclidine, alone or in combination with the traditional antidotes composed of atropine and 2-pralidoxime, against the poisoning with major nerve-agents such as tabun (*N*-dimethylphosphoramidocyanidate), sarin, soman, cyclosarin (cyclohexylmethylphosphonofluoridate) and V-agent (*O*-ethyl-S-[2-(diisopropylamino)ethyl]methylphosphonothioate).

II. MATERIALS AND METHODS

1. Materials

Physostigmine salicylate, atropine sulfate, procyclidine hydrochloride and 2-pralidoxime methochloride were procured from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Organophosphates tabun, sarin, cyclosarin, soman and V-agent were synthesized in Chemical Analysis Laboratory of Agency for Defense Development.

2. Animals

Specific pathogen-free Sprague-Dawley female rats (body weight, 200~250 g) and conventional Dunkin-Hartly male guinea pigs (body weight, 250~300 g) were housed in an environmentally-controlled room with temperature of 23 2, relative humidity of 55 5%, a 12-hr light/dark cycle, and feed and water available *ad libitum*.

3. Antidotal efficacy

Protective effect of each combination on the lethality of animals intoxicated with nerve-agents (tabun, sarin, soman, cyclosarin or V-agent) was expressed as protection ratio (fold of median lethal dose [LD_{50}] in treated group over LD_{50} in control group). The prophylactics, physostigmine (0.1 mg/kg) in combination with procyclidine (0~6.0 mg/kg), were administered subcutaneously 30 min prior to subcutaneous injec-

tion of each nerve-agent (Kim *et al.*, 1998). Separately, the traditional antidotes composed of atropine (17.4 mg/kg) and 2-pralidoxime (30 mg/kg), alone or in combination with the prophylactic regimen, were treated intramuscularly immediately after the challenge with nerve-agents. All the therapeutics were dissolved in physiological saline and administered in a volume of 1 ml/kg. Nerve-agents were dissolved in 10% isopropyl alcohol (10 mg/ml) to make a stock solution which was stored at 4°C, further diluted in physiological saline immediately before use, and administered in a volume of 1 ml/kg. The LD_{50} values were estimated based on 24-hr mortalities in each group of animals given increasing dose levels according to the method of Litchfield and Wilcoxon (1949).

The experiments performed here were conducted according to the *"Guide Principles in the Use of Animals in Toxicology"* which had been adopted by the Society of Toxicology in 1989.

III. RESULTS

The LD_{50} values of tabun, sarin, soman, cyclosarin and V-agent administered subcutaneously to rats were determined to be 255, 130, 75, 160 and 14 μg/kg, respectively, and that of soman in guinea pigs was 35 μg/kg, indicative of a higher sensitivity of guinea pigs to soman than rats (Table 1).

To assess the dose-response relationship in rats, various doses (0.3~6.0 mg/kg) of procyclidine in combination with a fixed dose (0.1 mg/kg) of physostigmine were pretreated subcutaneously 30 min prior to subcutaneous poisoning with each nerve-agent. Physostigmine (0.1 mg/kg) alone exerted protection ratios of 2.44, 1.20, 1.50, 1.50 and 2.20 folds for tabun, sarin, soman, cyclosarin and V-agent, respectively. Interestingly, coadministration of procyclidine with physostigmine (0.1 mg/kg) synergistically enhanced the protection ratio in a dose-dependent manner, leading to 4.00, 5.70, 7.20 and 8.00 folds for tabun, 2.15, 2.85, 6.50 and 8.50 folds for sarin, 1.92, 2.24, 3.95 and 5.07 folds for soman, 2.15, 2.00, 2.90 and 2.80 folds for cyclosarin, and 2.71, 3.86, 8.00 and 10.50 folds for V-agent with 0.3, 1.0, 3.0 or 6.0 mg/kg of procyclidine, respectively (Table 1 and Fig. 1).

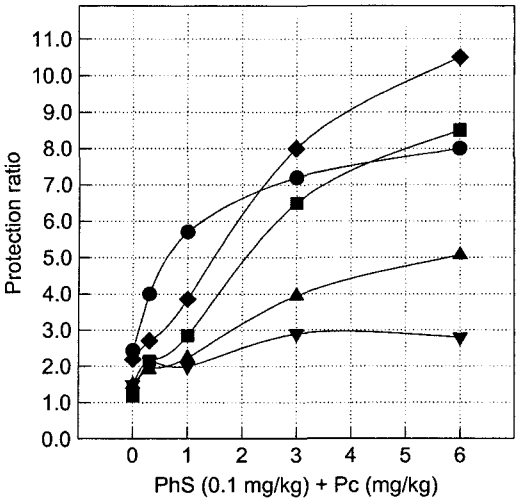

Fig. 1. Dose-response curve of procyclidine in combination with physostigmine (0.1 mg/kg) in the protection ratio (folds of control LD_{50}) against nerve-agent poisoning in rats. PhS, physostigmine; Pc, procyclidine; ●, tabun; ■, sarin; ▲, soman; ▼, cyclosarin; ◆, V-agent.

In addition, the prophylactic treatment with physostigmine (0.1 mg/kg) and procyclidine exerted protective effects of 3.00, 3.25, 4.50 and 4.70 folds with 0.3, 1.0, 3.0 and 6.0 mg/kg of procyclidine, respectively, against soman poisoning in guinea pigs, which were relatively higher at low doses (0.3~3.0 mg/kg) of procyclidine, but rather lower at a high dose (6.0 mg/kg) of procyclidine than those in rats (Fig. 2).

Table 1. Effect of physostigmine (0.1 mg/kg) alone or in combination with procyclidine (0.3~6.0 mg/kg) on the median lethal dose (LD_{50}, μg/kg) of nerve-agents. Physostigmine and procyclidine were pretreated subcutaneously 30 min prior to subcutaneous poisoning with each nerve-agent

Treatment (mg/kg)	tabun	sarin	soman		cyclosarin	V-agent
	rat	rat	rat	guinea pig	rat	rat
Vehicle	255	130	75	35	160	14
Physostigmine (0.1) alone	623	156	113	63	240	31
+ Procyclidine (0.3)	1,020	280	144	97	344	38
+ Procyclidine (1.0)	1,454	370	168	105	320	54
+ Procyclidine (3.0)	1,836	845	296	140	464	112
+ Procyclidine (6.0)	2,040	1,105	380	158	432	147

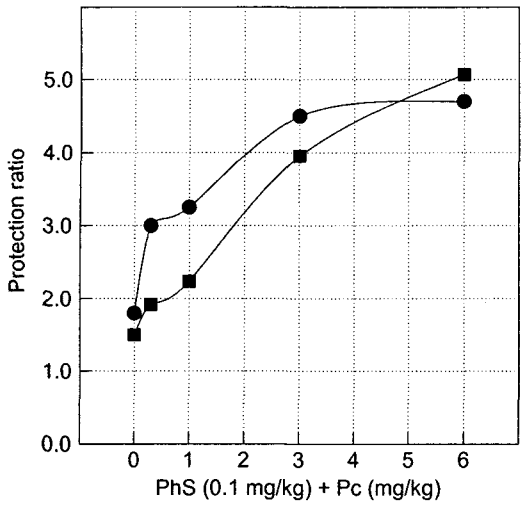

Fig. 2. Dose-response curve of procyclidine in combination with physostigmine (0.1 mg/kg) in the protection ratio (folds of control LD_{50}) against soman poisoning in rats (■) and guinea pigs (●). PhS, physostigmine; Pc, procyclidine.

Table 2. Protective effect of combinational antidotes against soman poisoning. Physostigmine (PhS) and procyclidine (Pc) were pretreated subcutaneously 30 min prior to subcutaneous poisoning with soman, followed by immediate intramuscular injection with atropine sulfate (AtSO4) and 2-pralidoxime (2-PAM)

Treatment (mg/kg)	Protection ratio (folds)
PhS (0.1)	1.50
Pc (3.0)	1.50
PhS (0.1) + Pc (1.0)	2.24
PhS (0.1) + Pc (3.0)	3.95
AtSO4 (17.4) + 2-PAM (30)	1.65
PhS (0.1) + Pc (1.0) + AtSO4 (17.4) + 2-PAM (30)	6.13
PhS (0.1) + Pc (3.0) + AtSO4 (17.4) + 2-PAM (30)	12.27

For further investigation on the combinational effects of the prophylactics and traditional antidotes used in field, physostigmine and procyclidine were treated prior to soman challenge followed by atropine and 2-pralidoxime. Posttreatment with atropine (17.5 mg/kg) and 2-pralidoxime (30 mg/kg) increased the LD_{50} value of soman by only 1.65 fold (Table 2). In contrast, the protection ratio of the antidotes was greatly enhanced to 6.13 and 12.27 folds by pretreatment with physostigmine (0.1 mg/kg) and procyclidine at doses of 1.0 and 3.0 mg/kg, respectively. Moreover, most of the animals survived soman challenge after pretreatment with physostigmine and procyclidine recovered within 4 hr without severe physical incapacitation, in contrast to severe convulsions and incoordination of rats received only atropine and 2-pralidoxime.

IV. DISCUSSION

The most toxic nerve-agent subcutaneously administered to rats was V-agent, followed by soman, sarin, cyclosarin and tabun (Table 1). Pretreatment of physostigmine alone remarkably enhanced the LD_{50} values of tabun (2.44 fold) and V-agent (2.20 fold), although it increased that of sarin (1.20 fold) to a small extent. Interestingly, it was reported that centrally-inactive pyridostigmine not only rather potentiated the toxicity of sarin only (Gordon *et al.*, 1978), but also reduced the antidotal efficacy of atropine and 2-pralidoxime against sarin and V-agent poisoning when administration of the antidotes were delayed (Koplovitz *et al.*, 1992b). Noteworthy, great synergistic protective effects were achieved with the combinational pretreatment with physostigmine and procyclidine in the poisoning with tabun, sarin and V-agent, compared to the relatively-low efficacies against cyclosarin and soman poisoning. In addition, the animals pretreated with physostigmine and procyclidine at low doses (1.0~3.0 mg/kg), followed by posttreatment with atropine and 2-pralidoxime, rapidly recovered from soman poisoning, resulting in high protection ratios (6.13~12.27 folds). The relatively-low efficacy of physostigmine and procyclidine against cyclosarin may be somewhat in parallel with the low effects of pyridostigmine, atropine and 2-pralidoxime in rodents (Clement, 1992; Lundy *et al.*, 1992), in contrast to a high effect in monkeys (Koplovitz *et al.*, 1992a). Accordingly, additional investigations on the combinational effects with other antidotes and the species differences remain to be clarified.

Unfortunately, it is possible that the standard field regimen, pretreatment with pyridostigmine tablet and posttreatment with atropine, 2-pralidoxime and diazepam injection, not only make soldiers or farmers confused but also fail to exert enough antidotal and neuroprotective efficacies in emergency situations. In the present study, we investigated the prophylactic efficacy of physostigmine plus procyclidine as a basic research in order to develop a soldiers' or farmers' patch containing the drugs. More recently, we have confirmed that physostigmine and procyclidine readily penetrated the skin enough to reach the blood con-

centration achieved from the effective injection doses (unpublished data). The simple prescription consisted of only 2 compounds, physostigmine and procyclidine, might cover the pharmacological actions of the previous 4 drugs without delay of treatment, as inferred from the high antidotal, anticonvulsant and neuroprotective effects (Kim *et al.*, 1997, 1998, 2000, present study). In addition, it was expected that the possible adverse effects of carbamates and anticholinergics used prophylactically might be offset by each other (Berry and Davies, 1970; Lim *et al.*, 1991; Philippens *et al.*, 2000). For example, the doses of procyclidine influencing the physical and physiological functions of rats were increased by the combination with physostigmine (unpublished data). Moreover, anticholinergics including procyclidine could recover the respiratory suppression induced by diazepam and dizocilpine (MK-801), a non-competitive *N*-methyl-D-aspartate antagonist, during organophosphate poisoning (McDonough and Shih, 1993; Shih, 1990), and prevent the neurotoxicity of dizocilpine (Hur *et al.*, 1999; Olney *et al.*, 1991).

In the present study, it was confirmed that the combinational prophylactics composed of physostigmine and procyclidine exerted great synergistic protective effects against major organophosphates of militarily importance, and further enhanced the efficacy of standard antidotes. In is of interest to note that the efficacy of carbamates in combination with anticholinergics was high in monkeys followed by guinea pigs, dogs, rabbits, mice, chickens and rats (Berry and Davies, 1970; Dirnhuber *et al.*, 1979; Gordon *et al.*, 1978). In our results, the efficacy of physostigmine (0.1 mg/kg) alone or in combination with low doses (0.3~3.0 mg/kg) of procyclidine in guinea pigs was much higher than that in rats. Thus, it is expected that a remarkable effect could be achieved with physostigmine and procyclidine in primates including human. Taken together, it is suggested that physostigmine plus procyclidine could be a promising prophylactic regimen for the poisoning with organophosphates possessing diverse properties.

REFERENCES

Berman, H.A. and Decker, M.M. (1986): Kinetic, equilibrium, and spectroscopic studies on dealkylation ("aging") of alkyl organophosphonyl acetylcholinesterase, *J. Biol. Chem.*, **261**, 10646-10652.

Berry, W.K. and Davies, D.R. (1970): The use of carbamates and atropine in the protection of animals against poisoning by 1,2,2-trimethylpropylmethylphosphonofluoridate, *Biochem. Pharmacol.*, **19**, 927-934.

Clement, J.G. (1992): Efficacy of various oximes against GF (cyclohexylmethylphosphonofluoridate) poisoning in mice, *Arch. Toxicol.*, **66**, 143-144.

Clement, J.G. and Broxup, B. (1993): Efficacy of diazepam and avizafone against soman-induced neuropathology in brain of rats, *Neurotoxicology*, **14**, 485-504.

Dirnhuber, P., French, M.C., Green, D.M., Leadbeater, L. and Stratton, J.A. (1979): The protection of primates against soman poisoning by pretreatment with pyridostigmine, *J. Pharm. Pharmacol.*, **31**, 295-299.

Dunn, M.A. and Sidell, F.R. (1989): Progress in medical defense against nerve agents, *JAMA*, **262**, 649-652.

Gao, Z.G., Liu, B.Y., Cui, W.Y., Li, L.J., Fan, Q.H. and Liu, C.G. (1998): Anti-nicotinic properties of anticholinergic antiparkinson drugs, *J. Pharm. Pharmacol.*, **50**, 1299-1305.

Gordon, J.J., Leadbeater, L. and Maidment, M.P. (1978): The protection of animals against organophosphate poisoning by pretreatment with a carbamate, *Toxicol. Appl. Pharmacol.*, **43**, 207-216.

Harris, L.W., McDonough, J.H., Stitcher, D.L. and Lennox, W.J. (1984): Protection against both lethal and behavioral effects of soman, *Drug Chem. Toxicol.*, **7**, 605-624.

Hur, G.-H., Son, W.-C., Shin, S., Kang, J.-K. and Kim Y.-B. (1999): Sex differences in dizocilpine (MK-801) neurotoxicity in rats, *Environ. Toxicol. Pharmacol.*, **7**, 143-146.

Keeler, J.R., Hurst, C.G. and Dunn, M.A. (1991): Pyridostigmine used as a nerve agent pretreatment under wartime conditions, *JAMA*, **266**, 693-695.

Kim, Y.-B., Hur, G.-H., Lee, Y.-S., Han, B.-G. and Shin, S. (1997): A role of nitric oxide in organophosphate-induced convulsions, *Environ. Toxicol. Pharmacol.*, **3**, 53-56.

Kim, Y.-B., Hur, G.-H., Shin, S., Sok, D.-E., Kang, J.-K. and Lee Y.-S. (1999): Organophosphate-induced brain injuries: delayed apoptosis mediated by nitric oxide, *Environ. Toxicol. Pharmacol.*, **7**, 147-152.

Kim, Y.-B., Hur, G.-H., Shin, S., Yeon, G.-B., Choi, S.-J. and Kang, J.-K. (2000): Prophylactic detoxification by physostigmine and procyclidine of diisopropylfluorophosohate poisoning, *J. Toxicol. Pub. Health*, in press.

Kim, Y.-B., Shin, S., Sok, D.-E. and Kang, J.-K. (1998): Effectiveness of procyclidine in combination with carbamate prophylactics against diisopropylfluorophos-

phate poisoning, *Environ. Toxicol. Pharmacol.*, **5**, 43-49.

Koplovitz, I., Gresham, V.C., Dochterman, L.X., Kaminskis, A. and Stewart, J.R. (1992a): Evaluation of the toxicity, pathology, and treatment of cyclohexylmethylphosphonofluoridate (CMPF) poisoning in rhesus monkeys, *Arch. Toxicol.*, **66**, 622-628.

Koplovitz, I., Harris, L.W., Anderson, D.R., Lennox, W.J. and Stewart, J.R. (1992b): Reduction by pyridostigmine pretreatment of the efficacy of atropine and 2-PAM treatment of sarin and VX poisoning in rodents, *Fund. Appl. Toxicol.*, **18**, 102-106.

Lallement, G., Carpentier, P., Collet, A., Pernot-Marino, I., Baubichon, D. and Blanchet, G. (1991): Effects of soman-induced seizures on different extracellular amino acid levels and glutamate uptake in rat hippocampus, *Brain Res.*, **563**, 234-240.

Lallement, G., Clarencon, D., Masqueliez, C., Baubichon, D., Galonnier, M., Burckhart, M.-F., Peoc'h, M. and Mestries, J.C. (1998): Nerve agent poisoning in primates: antilethal, anti-epileptic and neuroprotective effects of GK-11, *Arch. Toxicol.*, **72**, 84-92.

Lim, D.K., Hoskins, B. and Ho, I.K. (1991): Trihexylphenidyl enhances physostigmine prophylaxis against soman poisoning in guinea pigs, *Fund. Appl. Toxicol.*, **16**, 482-489.

Litchfield, J.T. and Wilcoxon, F. (1949). A simple method of evaluating dose-effect experiments, *J. Pharmacol. Exp. Ther.*, **96**, 99-115.

Lundy, P.M., Hansen, A.S., Hand, B.T. and Boulet, C.A. (1992): Comparison of several oximes against poisoning by soman, tabun and GF, *Toxicology*, **72**, 99-105.

Mattson, M.P. (1989): Acetylcholine potentiates glutamate-induced neurodegeneration in cultured hippocampal neurons, *Brain Res.*, **497**, 402-406.

McDonough, J.H. and Shih, T.-M. (1995): A study on the *N*-methyl-D-aspartate antagonistic properties of anticholinergic drugs, *Pharmacol. Biochem. Behav.*, **51**, 249-253.

McDonough, J.H. and Shih, T.-M. (1993): Pharmacological modulation of soman-induced seizures, *Neurosci. Biobehav. Rev.*, **17**, 203-215.

McDonough J.H., Zoeffel L.D., McMonagle, J., Copeland, T.L., Smith, C.D. and Shih, T.-M. (2000): Anticonvulsant treatment of nerve agent seizures: anticholinergics versus diazepam in soman-intoxicated guinea pigs, *Epilepsy Res.*, **38**, 1-14.

Nagao, M., Takatori, T., Matsuda, Y., Nakajima, M., Iwase, H. and Iwadate, K. (1997): Definitive evidence for the acute sarin poisoning diagnosis in the Tokyo subway, *Toxicol. Appl. Pharmacol.*, **144**, 198-201.

Olney, J.W., Labruyere, J., Wang, G., Wozniak, D.F., Price, M.T. and Sesma, M.A. (1991): NMDA antagonist neurotoxicity: mechanism and prevention, *Science*, **254**, 1515-1518.

Philippens, I.H.C.H.M., Melchers, B.P.C., De Groot, D.M.G. and Wolthuis, O.L. (1992): Behavioral performance, brain histology, and EEG sequela after immediate combined atropine/diazepam treatment of soman-intoxicated rats, *Pharmacol. Biochem. Behav.*, **42**, 711-719.

Philippens, I.H.C.H.M., Vanwersch, R.A.P., Groen, B., Oliver, B., Bruijnzeel, P.L.B. and Melchers, B.P.C. (2000): Subchronic physostigmine pretreatment in marmosets: Absence of side effects and effectiveness against soman poisoning with negligible postintoxication incapacitation, *Toxicol. Sci.*, **53**, 84-91.

Shih, T.-M. (1990): Anticonvulsant effects of diazepam and MK-801 in soman poisoning, *Epilepsy Res.*, **7**, 105-116.

Shih, T.-M., Koviak, T.A. and Capricio, B.R. (1991): Anticonvulsants for poisoning by the organophosphorus compound soman: pharmacological mechanisms, *Neurosci. Biobehav. Rev.*, **15**, 349-362.

Shih, T.-M., McDonough, J.H. and Koplovitz, I. (1997): Evaluation of anticonvulsant drugs for soman-induced seizure activity, *J. Am. College Toxicol.*, **15**, S43-S60.

Somani, S.M. (1992): Chemical Warfare Agents. Academic Press, San Diego, pp. 67-207.

Suzuki, T., Morita, H., Ono, K., Maekawa, K., Nagai, R. and Yazaki, Y. (1995): Sarin poisoning in Tokyo subway, *Lancet*, **345**, 980-981.

Talbot, B.G., Anderson, D.R., Harris, L.W., Yarbrough, L.W. and Lennox, W.J. (1988): A comparison of *in vivo* and *in vitro* rates of aging of soman-inhibited erythrocyte acetylcholinesterase in different animal species, *Drug Chem. Toxicol.*, **11**, 289-305.

Tryphonas, L. and Clement, J.G. (1995): Histomorphogenesis of soman-induced encephalocardiomyopathy in Sprague-Dawley rats, *Toxicol. Pathol.*, **23**, 393-409.

Tryphonas, L., Veinot, J.P. and Clement, J.G. (1996): Early histopathologic and ultrastructural changes in the heart of Sprague-Dawley rats following administration of soman, *Toxicol. Pathol.*, **24**, 190-198.

Waelbroeck, M., Camus, J., Tastenoy, M., Lambrecht, G., Mutschler, E., Tacke, R. and Christophe, J. (1990): Stereoselectivity of procyclidine binding to muscarinic receptor subtypes M1, M2 and M4, *Eur. J. Pharmacol.*, **189**, 135-142.