Chapter 6

NEUROPROTECTION AS A TREATMENT FOR NERVE AGENT SURVIVORS

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INTRODUCTION

Organophosphorus nerve agents are the principal chemical warfare agents known to produce brain injury. They block hydrolysis of the neurotransmitter acetylcholine by inhibiting the enzyme acetylcholinesterase, resulting in greatly increased postsynaptic acetylcholine levels. This causes a spectrum of effects, including miosis, excess secretions, nausea, vomiting, and muscle fasciculations. At moderate to high doses, nerve agents also cause seizures and associated convulsions. If left untreated, seizures rapidly progress to status epilepticus (SE) and cause irreversible seizure-related brain damage (SRBD).[1,2] The International Classification of Epileptic Seizures defines SE as any seizure lasting at least 30 minutes or intermittent seizures lasting longer than 30 minutes between which the patient does not regain consciousness.[3,4]

For over a decade acute therapy has effectively saved those poisoned by nerve agents on the battlefield,[5] after accidental exposures,[6] and in terrorist attacks, as in the Japan subway attacks in 1994 and 1995. One lesson learned from the 1995 Tokyo attack was that, lacking acute antidotal treatment, many survivors arrived at hospitals in convulsive SE. The Tokyo experience illustrates the necessity of acute antidotal therapy, such as the regimen adopted by the US military. This regimen is aimed primarily at treating cholinergic crisis with a postexposure anticholinergic (atropine sulfate) and an oxime reactivator (2-pralidoxime [2-PAM Cl]). In specific intelligence-driven situations, pyridostigmine bromide (PB) pretreatment is added. Although these medications greatly reduce morbidity and mortality, they do not always prevent seizures and brain damage in nerve agent casualties; therefore, the regimen now includes the anticonvulsant diazepam.[2]

Even with diazepam, however, the treatment regimen has limitations. The decision to include diazepam was based on animal data showing that it could terminate nerve-agent–induced seizures and convulsions and enhance survival when given in conjunction with the acute therapy described above.[7–11] However, the therapeutic window for arresting seizures and SE with diazepam is less than an hour following onset; after that, both are refractory to anticonvulsant therapy.[7,8,10–19]

Early use of an anticonvulsant does not guarantee that seizures, once stopped, will not return. The recurrence of seizures is often observed in animal studies in several species and is of concern in human exposures. Although neuropathology is reduced in diazepam-treated animals, the incidence and degree of protection afforded by diazepam is not complete.[9,20–23] Moreover, switching the fielded anticonvulsant to another benzodiazepine, such as midazolam or lorazepam, does not entirely solve the problem of refractory SE.

Seizures and SE are key causes of brain damage resulting from nerve agent poisoning, and their prevention or alleviation should be the primary objective.[24–26] However, because of the refractory nature of seizures and especially SE, prevention and alleviation become increasingly difficult as more time elapses before therapy begins. Also, there is high probability that seizures will return when anticonvulsants wear off. Therefore, it is reasonable to anticipate a high incidence of brain damage connected to the increased survival rate of nerve agent victims.

Casualties exhibiting seizures and SE can be anticipated not only from terrorist attacks but also from battlefield scenarios involving troops who were not in full protective ensemble at the time of the attack.[27] In the confusion following a terrorist attack or on the battlefield, prompt treatment of nerve agent casualties can be expected to be problematic, and some victims undergoing seizures may not receive anticonvulsants inside the antiseizure therapeutic window. It is also possible that some victims may undergo nonconvulsive SE, a state of continuous seizures without observable clinical movement.[28] For these victims, treatment might be inadvertently delayed beyond the therapeutic window. Under the Small Business Innovative Research Program, the US Army funds efforts to field a far-forward, simple seizure detector to identify these casualties.

This chapter presents a detailed overview of nerve-agent–induced neuropathology and explains the mechanisms of action of candidate neuroprotectants that have shown promise in various animal and human studies, especially those that have received US Food and Drug Administration (FDA) approval for other indications.

NEUROPATHOLOGY AND THE MECHANISM OF NERVE-AGENT–INDUCED DAMAGE

Although there is little neuropathological data from patients who have survived nerve agent attacks, abundant evidence is available from animal models, many of which involve persistent SE. The profound brain damage produced by nerve agents was first described by Petras[29]; Lemercier et al[30]; and McLeod et al.[31] Since then, numerous studies have greatly enhanced the understanding of neuropathology resulting from nerve agent intoxication.[23,32–38] These studies have established that prolonged seizures and SE resulting

from nerve agent exposure are directly responsible for the vast majority, if not all, of the neuropathology produced by these agents. The associated damage is typically bilaterally symmetrical and most severe in temporal lobe structures (ie, piriform and entorhinal cortices, hippocampus, and amygdala) as well as in the thalamus.

Brain damage resulting from agent-induced seizures is the result of the complex, multiphasic response of individual neurons to numerous extracelluar and intracellular events. Following inhibition of acetylcholinesterase and accumulation of acetylcholine at cholinergic synapses, the hyperstimulation of cholinergic receptors on postsynaptic membranes triggers seizures.[10,39,40] Subsequently, recruitment and excessive activation of the glutamatergic neurotransmitter system occurs. Glutamate, the most abundant excitatory neurotransmitter in the brain, is responsible for sustaining soman-induced seizures and promoting the development of SE.[1,24,41–44] Large pathological elevations in the concentration of intracellular sodium and (especially) calcium are caused by excessive stimulation of ionotropic glutamate receptors, as is prolonged depolarization of postsynaptic membranes. This initiates a harmful cascade of pathological processes, most of which center around a prolonged increase in intracellular free calcium or delayed calcium overload, leading to excitotoxic cell death.[1,24,45–47]

Transient elevation in intracellular free calcium is a ubiquitous signaling mechanism and regulator of intracellular processes, from cell growth and metabolism to cell death.[48–50] Cytosolic free calcium is also a critical neuronal mediator of learning and memory.[51] However, when normal homeostatic control of intracellular calcium is lost and a sustained elevation occurs, the delayed calcium overload triggers neuronal cell death by necrosis or apoptosis (a form of programmed cell death).[52–56] In neurons, the majority of calcium influx occurs through *N*-methyl D-aspartate (NMDA) ionotropic glutamate receptors as well as voltage-gated calcium channels (eg, L-type). Calcium influx also occurs, though to a lesser extent, through the other two classes of ionotropic glutamate receptors (alpha-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid and kainate receptors).[57] Excessive stimulation of NMDA receptors is the first step in glutamate excitotoxicity.[24,45]

The release of intracellular stores is also responsible for increased cytosolic free calcium. The endoplasmic reticulum (ER) releases calcium following binding of the second messenger, inositol triphosphate, to ionotropic receptors located on the ER membrane. Calcium is released from the ER via ryanodine receptors. These ionotropic receptors are also located on the ER membrane and open following binding of cytosolic calcium; thus, cytosolic free calcium augments its own concentration by stimulating calcium release from the ER.[49] The ER plays a critical role in normal calcium homeostasis. Excessive release or impaired uptake of calcium has been implicated in pathology resulting from calcium overload.[49,52] Brain mitochondria are important for calcium buffering as cytosolic concentrations rise, and their ability to sequester calcium is dependent on adenosine triphosphate (ATP).[58] However, when calcium overload occurs, mitochondria undergo a permeability transition characterized by loss of mitochondrial transmembrane potential, curtailment of ATP synthesis, mitochondrial swelling, release of stored calcium, and neuronal death by necrosis.[59–62]

The majority of soman-induced SRBD results from glutamate excitotoxicity and the delayed calcium overload that follows.[1,24,42,43] Delayed calcium overload in neurons initiates a pathological sequence characterized by activation of several potentially damaging enzymes. These include oxygenases, phospholipases, and nitric oxide synthase, which produce reactive oxygen species such as superoxide radical, hydrogen peroxide, hydroxyl radical, nitric oxide, and peroxynitrite. Neuronal injury induced by reactive oxygen species stems from direct damage to cell membranes, DNA, and intracellular proteins, and also induction of cytochrome C from mitochondria with subsequent caspase activation.[62] Release of cytochrome C, caspase activation, and DNA fragmentation are molecular hallmarks of apoptosis (Figure 6-1).[56,62,63]

Cysteine proteases called calpains are also activated by sustained elevations in intracellular free calcium. Calpains degrade various intracellular proteins, including those of the cytoskeleton, membrane channels, and metabolic enzymes, and cause neuronal death by necrosis.[56,62,63] (Necrosis produces localized inflammation, which exacerbates damage, while apoptosis is not associated with inflammation.) The culmination of these events may result in cell death hours or days after the initial insult.[53–55]

Necrosis and apoptosis are not an either/or phenomena, that is, they are not completely distinct forms of cell death with no overlap; a necrosis versus apoptosis dichotomy is a misleading over-simplification.[64,65] Martin and colleagues proposed an "apoptosis-necrosis continuum," reporting that dying neurons can exhibit intermediate forms between apoptosis and necrosis.[66] Recently, Baille and colleagues confirmed that neuronal injury, resulting from soman-induced seizures, exhibits a large variety of hybrid forms between necrosis and apoptosis, but that the majority show more necrotic features.[67] Whether soman-induced neuropathology is mostly necrotic, as it is in the piriform cortex of rats,[38] or contains elements of apoptosis as first proposed

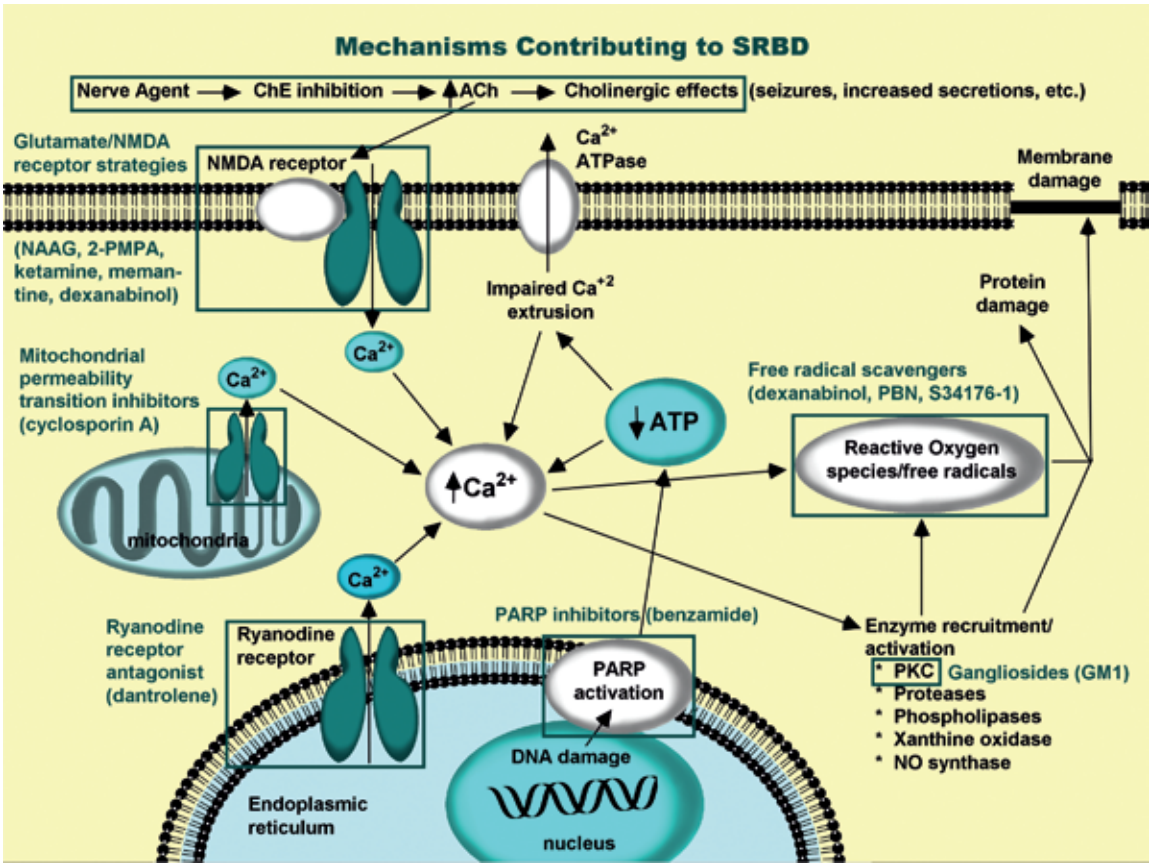

Fig. 6-1. Mechanisms contributing to nerve agent-induced SRBD. Calcium plays a pivotal role in glutamate excitotoxicity. A number of pharmacological approaches to neuroprotection have been investigated. Various sites in this pathway have been targeted. NMDA receptor antagonists block calcium entry through this glutamate ionotropic receptor. Gangliosides promote calcium extrusion indirectly by blocking PKC translocation (not indicated). PARP inhibitors enhance functionality of ion pumps and calcium extrusion by increasing ATP availability. Dantrolene blocks calcium release from intracellular stores. Free radical scavengers include free radical "traps" and endogenous free radical scavenging enzymes and small molecules prevent oxidative damage.

by Ballough et al in 1997 and definitively assessed by Baille et al is less important than the fact that both forms of neuronal cell death are triggered by nerve-agent–induced seizures.[38,67,68]

Candidate drugs may alter the relative proportions of neurons undergoing death by necrosis versus apoptosis. Studies have reported that insufficient ATP availability is an important determinant of whether a cell that has been triggered to undergo apoptosis is instead forced to die by necrosis.[55,69,70] Therefore, it is conceivable that a neuroprotectant candidate that enhances ATP availability (for example, poly(ADP-ribose) polymerase [PARP] inhibitors) could suppress necrosis while facilitating apoptosis. Neither possibility should be excluded during pathological evaluations of neuroprotectant candidates.

SPECIFIC RELEVANCE OF NEUROPROTECTION TO NERVE AGENT SURVIVORS

The term "neuroprotection" is defined as "pharmacological intervention that produces enduring benefits by favorably influencing underlying etiology or pathogenesis and thereby forestalling the onset of disease or clinical decline."[71,72] Within this broad definition, neuroprotection has acquired many different connotations. As a result, a search of the term "neuroprotection" on the National Library of Medicine's PubMed search page produces several thousand studies, mostly on disease states in which subsets of neurons are specifically vulnerable and die prematurely (as happens in Parkinson's disease, Huntington's disease, frontotemporal dementia, and a host of metabolic disorders) or accumulate neuropathology seen to a slight degree in

normal brains but in an accelerated fashion in some diseases (such as Alzheimer's disease and trisomy 21). However, such interventions are unlikely to be relevant to the survivor of a single, brief nerve agent exposure that has already caused sustained seizures and SE. On the other hand, research on neuroprotection following stroke has provided valuable insights and clues that do apply to the nerve agent survivor.

In this chapter, the term "neuroprotection" specifically refers to a putative intervention given over a short period, ideally closely following the diagnosis of nerve agent exposure or before the acute toxic syndrome of exposure has been adequately treated. The best neuroprotectant would have the longest therapeutic window during which administration would be beneficial (even if the window is still only a matter of hours). At the same time, for logistical and doctrinal reasons, the neuroprotection initiative does not extend to prophylactic treatments administered to troops likely to experience nerve agent exposure (which would constitute a pretreatment, such as the bioscavenger initiative [see Chapter 7, Nerve Agent Bioscavenger: Development of a New Approach to Protect Against Organophosphorus Exposure]). Therefore, in this chapter, neuroprotection refers only to postexposure treatment.

There are similarities between brain damage resulting from nerve-agent–induced seizures and secondary neuronal injury resulting from stroke.[73,74] Although the immediate aspect of stroke-related neuronal injury is necrosis, which stems from anoxia or hypoxia, there is a secondary component to stroke damage that takes 48 to 72 hours to become manifest. This component accounts for approximately 50% of the total damage resulting from the ischemic episode. Secondary stroke injury involves brain tissue immediately surrounding the necrotic core of primary injury (the penumbra). For the most part, glutamate excitotoxicity and ionic destabilization, especially intracellular calcium, induce penumbral damage.[73–75] Thus, the similarities between secondary stroke damage and damage resulting from nerve-agent–induced seizures become apparent: they both involve glutamate excitotoxicity, hinge on intracellular calcium destabilization, and lead to necrotic or apoptotic neuronal death. This similarity raises the possibility that neuroprotectants being developed for stroke may be useful for nerve agent survivors. Neuroprotective interventions in stroke models have been shown to save neurons that otherwise would have died via necrosis or apoptosis. There is hope, then, that a treatment can be found that can be administered after agent exposure and that, although it may not have any immediately discernible clinical effect, will produce a significantly improved long-term neurological outcome. Any of the many classes of compounds that have been suggested as acute stroke neuroprotectant candidates could be tried. This list is extensive; the Internet Stroke Center (http://www.strokecenter.org), maintained by Washington University,[76] offers a continuously updated list of compounds that have been tried in clinical stroke trials.

The rationale for developing a protective agent, especially one based on dissimilar clinical situations that give rise to similar neuronal pathology, assumes that preventing neuronal loss will produce a superior clinical outcome. In the case of stroke, this assumption is probably warranted. In the case of nerve-agent–induced nerve cell damage, this assumption has never been tested directly, but it is consistent with a wide variety of animal data in multiple models and species. The assumption that preventing brain damage will produce superior behavioral outcome is even supported by Lashley and Hebb's studies in the early to mid 1900s.[77] A neuroprotectant in this restricted sense should demonstrate that neurons that might have been lost are now saved and that behavioral or neurological outcome is improved. An ideal database to document such neuroprotectants would include both neuropathological evidence of neuron survival and behavioral (in animals) or cognitive (in people) evidence that the neurologic outcome is superior compared to subjects that did not receive the neuroprotectant. Finally, the FDA must approve use of the agent if it is a medication. (In clinical medicine, any FDA-approved medication can be used off-label by licensed physicians, but in military doctrine, specific on-label FDA approval is mandatory.)

NEUROPROTECTANTS WITH PROVEN EFFICACY AGAINST NERVE-AGENT–INDUCED SIEZURE-RELATED BRAIN DAMAGE

This research comes from the consensus that nerve-agent–induced seizures and SE lead to the development of glutamate-mediated excitotoxicity, in which delayed calcium overload is the intracellular trigger of the final sequences leading to cell death.[1,24,42,43,47,49,56,78–81] Classes of drugs that have been tested for their abilities to ameliorate nerve-agent–induced SRBD by specifically mitigating delayed calcium overload include the following:

- NMDA receptor antagonists that block extracellular calcium influx;
- glycosphingolipids that reduce intracellular calcium by blocking the translocation of

protein kinase C (PKC), thus enhancing the sodium-calcium exchange;
- ryanodine receptor antagonists that prevent the release of calcium from the ER; and
- PARP inhibitors that indirectly lower intracellular calcium by preventing ATP depletion.[82–89]

Increased ATP availability facilitates calcium efflux by plasma membrane Ca2+ ATPase and calcium sequestration by the mitochondria, and indirectly enhances sodium-calcium exchange by maintaining sodium-potassium-ATPase functionality.[58]

Gangliosides

Medications that target events subsequent to calcium overload have been tested against soman-induced SRBD in an effort to circumvent neurotoxicity associated with NMDA receptor antagonism and mitigate established delayed calcium overload. Intracerebroventricular infusion of GM1 monosialoganglioside (5 mg/kg/day, for 5 days before and 27 h after soman exposure) in rats markedly reduced cross-sectional areas of soman-induced temporal lobe necrosis (there was an 85.9% lesion reduction in the piriform cortex and contiguous structures, compared with unprotected soman-positive controls).[90] In this study, all rats were pretreated with PB before soman exposure, and then treated with atropine methylnitrate (AMN) and 2-pralidoxime (2-PAM). Considerable neuroprotection was also obtained with the water-soluble GM1 monosialoganglioside derivative, WILD20. As an adjunct to HI-6 pretreatment and AMN posttreatment, WILD20 (2.5 mg/kg, intraperitoneal injection [IP]) reduced volumetric temporal lobe necrosis by 75.2%. Neuroprotection by these two compounds occurred, and neither seizure intensity nor duration (assessed via electroencephalography [EEG] monitoring) was diminished.

Gangliosides are sialic-acid–containing glycosphingolipids that are natural constituents of cell membranes and are particularly abundant in neurons.[91–93] The mechanism by which GM1 monosialoganglioside and WILD20 exert their neuroprotective effects involves inhibition of PKC translocation to the plasma membrane.[75, 82–86,94,95] PKC activation and translocation enhance glutamate excitotoxicity.[96,97] Furthermore, PKC's role in the excitotoxic process is to prolong NMDA receptor activation and possibly inhibit calcium extrusion mechanisms.[82,75,98] In addition, WILD20 is reported to reduce inflammation by its inhibitory effects on specific leukocytes (neutrophils).[99] Despite the promising results with gangliosides, further studies have been discontinued because of concerns of possible contamination by prions associated with bovine spongiform encephalopathy (mad cow disease).[90,100]

Poly(ADP-ribose) Polymerase Inhibitors

Recent studies indicate that PARP inhibition is neuroprotective following neuropathological insults involving excitotoxicity, such as cerebral ischemia and traumatic brain injury.[101–108] PARP is an abundant nuclear enzyme that is activated by DNA strand breaks induced by reactive oxygen species.[108,109] With moderate insults, it facilitates DNA repair by utilizing cellular nicotinamide adenine dinucleotide to form poly(ADP-ribose). Excessive PARP activation leads to nicotinamide adenine dinucleotide depletion, metabolic inhibition via glycolysis block, ATP insufficiency, and cell death by necrosis.[104,109,110] Neurons are especially vulnerable to metabolic insufficiency resulting from PARP over-activation because glucose is normally the only metabolic substrate and the dependency on glycolysis is exceptionally high.[108] In excitotoxic models, over-activation of PARP is closely linked to calcium-induced nitric oxide synthase activation, which leads to the production of nitric oxide; the detrimental effects of nitric oxide are mostly mediated through peroxynitrite, which forms when nitric oxide reacts with superoxide.[109,111,112]

In 1999 Meier et al[113] reported reduced lesion volumes and increased survival in soman-exposed rats that received the PARP inhibitor benzamide. Further investigation into the neuroprotective efficacy of PARP inhibition warrants consideration, and subsequent studies should include several new-generation PARP inhibitors that have shown increased usefulness, such as ONO-1924H, DR2313, and FR247304.[105,107,114]

Ryanodine Receptor Antagonist

Dantrolene is another drug that has shown neuroprotective efficacy against soman-induced SRBD.[88] A ryanodine receptor antagonist that prevents the release of calcium from the ER, dantrolene is FDA-approved for use in malignant hyperthermia. Although some neuroprotection is produced by diazepam alone (20 mg/kg, intramuscular injection [IM], 40 min after seizure onset), this protection is significantly augmented in the dorsal and lateral cortices of rats by coadministration of dantrolene (10 mg/kg, intravenous [IV]).[88] Administering the full dosage of dantrolene in a single injection is difficult because of insolubility problems associated with the medication. To overcome these problems and achieve the desired dantrolene dosage, four separate IV injections were performed between

40 minutes and 8 hours after seizure onset, with a total injection volume approximating 1 mL per rat. A unique formulation of dantrolene (Lyotropic Therapeutics, Inc, Ashland, Va) as a nanocrystal dispersion has also been used to obviate solubility problems. With this formulation, it is possible to administer a much higher dose of dantrolene in a much lower injection volume. This is critical because when dantrolene is administered by IP injection, liver enzymes lower the concentration of dantrolene reaching the brain. The nanocrystal formulation of dantrolene minimizes the effects of the liver enzymes.

Our results with the dantrolene nanocrystal formulation not only overcame the insolubility problems of our previous dantrolene study, but corroborated and extended the results of that study. The nanocrystal study was unable to demonstrate significant protection in the piriform cortex, the most severely damaged region, but in this study the nanocrystal dispersion of dantrolene (40 mg/kg, IP) plus diazepam (20 mg/kg, IM) reduced piriform cortical necrosis by 15.6% more than diazepam alone (unpublished study by US Army Medical Research Institute of Chemical Defense). In these experiments, all soman-exposed rats also received HI-6 (125 mg/kg, IP, 30 min after soman) and AMN (2 mg/kg, IM, < 1 min after soman) to protect against the peripheral effects of soman and ensure survival. Neuroprotection by dantrolene in the above experiments occurred without changes in seizure intensity or duration, and dantrolene produced no discernible effects on the electrocorticographic profiles of soman-exposed subjects. These findings are consistent with those of Frandsen and Schouosboe,[115] who reported that dantrolene prevented glutamate neurotoxicity by blocking release of calcium from intracellular stores. The results are also consistent with those of Niebauer and Gruenthal,[87] who examined the protective effects of dantrolene on hippocampal neuronal damage produced by SE in rats. In their study, dantrolene (10 mg/kg, IP) was administered either 30 or 140 minutes after the onset of SE. Niebauer and Gruenthal reported that early administration produced a significant reduction in neuronal injury in all hippocampal subregions. When dantrolene administration was delayed until 140 minutes after SE onset, some protection was still seen in hippocampal field CA3, but not the other subregions.[87] Protection against kainic-acid–induced apoptosis has also been reported.[116]

N-methyl-D-aspartate Receptor Antagonists

MK-801 (Dizocilpine)

The first NMDA receptor antagonist to show promise as a putative neuroprotectant was MK-801 (dizocilpine); however, it has been shown to have toxic effects. When given in conjunction with PB, AMN, and 2-PAM, noncompetitive MK-801 was reported to reduce nerve-agent–induced SRBD in the piriform cortex, amygdala, hippocampus, and thalamus.[43] As mentioned, these are among the most severely damaged brain regions in SRBD resulting from soman exposure.[29–32,35,37,38,90] In the Sparenborg study, MK-801 (0.5, 1.0, or 5 mg/kg, IP) reduced brain damage and diminished or arrested seizures in guinea pigs when administered as a pretreatment 30 minutes before soman, and the effects were dose-dependent. The anticonvulsant profile of MK-801 against soman-induced seizures was definitively characterized by Shih.[11] He showed that the anticonvulsant effect of MK-801 is four times greater than that of diazepam, but at doses of 1 mg/kg or higher, MK-801 potentiated the lethal effects of soman. Some concern arose about the use of NMDA antagonists when it was reported that MK-801 induces neuronal degeneration in the posterior cingulate, retrosplenial cortices, and other corticolimbic regions.[117,118] This damage evidently occurs by disinhibition of multiple converging excitatory pathways.[119] Specifically, excessive blockage of glutamatergic pathways leads to excessive stimulation of cholinergic function.[120] This explanation is supported by the findings that neurotoxicity by MK-801 is augmented when cholinergic receptors (ie, muscarinic) are activated.[121]

Memantine

Memantine is a noncompetitive NMDA receptor antagonist[122] that has also been tested for its anticonvulsant effects against soman-induced seizures. Studies have suggested that memantine's pharmacokinetics make it a safer candidate than MK-801.[123,124] McLean et al[125] reported that memantine alone (18 mg/kg, subcutaneous [SC]) blocked the onset of soman-induced seizures and was able to terminate seizures when administered 15 minutes after soman injection. These findings, however, are inconsistent with those of Shih et al[17] who reported that memantine by itself is completely ineffective as an anticonvulsant against soman-induced seizures. The latter authors pointed to a need for EEG monitoring when determining anticonvulsant efficacy and suggested that McLean et al may have mistaken diminished convulsive behavior as evidence of reduced seizure activity. Neither study addressed the possible neuroprotective effects of memantine (ie, reduced neuropathology independent of anticonvulsant activity). On the other hand, Koplovitz et al[126] observed a modest reduction in piriform cortical damage following soman in rats treated with atropine and memantine, compared to those that received atropine alone. There were no differences between the

EEG power spectra of the two groups. Regardless of the above discrepancies, the neuroprotective benefit of memantine in other models of excitotoxicity is widely accepted.[124,127] For example, in a rat model of stroke, memantine given 2 hours after the ischemic event reduced brain damage by approximately 50%.[128] In addition, memantine is well tolerated and does not produce neurotoxicity at therapeutic dosages. It was recently approved by the FDA for treating Alzheimer's disease.[124]

HU-211 (Dexanabinol)

The first real proof of concept of postexposure neuroprotection came from work with HU-211 (dexanabinol), a nonpsychotropic analogue of tetrahydrocannabinol, the active ingredient in marijuana. Filbert and colleagues[129] showed that in rats exposed to high doses of soman, dexanabinol protected neurons in the piriform cortex (Figure 6-2) when given as late as 40 minutes after the EEG-proven onset of seizures. The drug was not an anticonvulsant and had no effect upon the seizures, indicating that the results showed a true neuroprotective effect and not part of an anticonvulsant effect. HU-211 has been reported to inhibit NMDA receptors, act as an antioxidant and free radical scavenger, suppress nitrous oxide and tumor necrosis factor-α generation, and stabilize calcium levels.[130–132] HU-211 is generally well tolerated in humans.[133]

When HU-211 (25 mg/kg, IP) was administered 5 minutes after the onset of soman-induced seizures, in conjunction with HI-6 and AMN pretreatment and posttreatment, respectively, temporal lobe lesion volume/necrosis (assessed at 28 h after seizure onset) was reduced by 86%, compared with unprotected soman-positive controls (see Figure 6-2).[134,135] HU-211 had no effect on the strength or duration of seizure activity, as determined by quantitative EEG analysis. Significant neuroprotection was also observed when HU-211 administration was delayed 40 minutes after seizure onset. Neuroprotection by HU-211 was most evident in the piriform cortex and contiguous temporal lobe structures, such as the amygdala, entorhinal, and perirhinal cortices, but did not extend to the thalamus. Administration of HU-211 and diazepam 40 minutes after seizure onset did not augment the neuroprotection obtained with diazepam alone.

In analyzing the mechanisms of neuroprotection by HU-211 and diazepam, it is important to differentiate between protection obtained by anticonvulsant effects

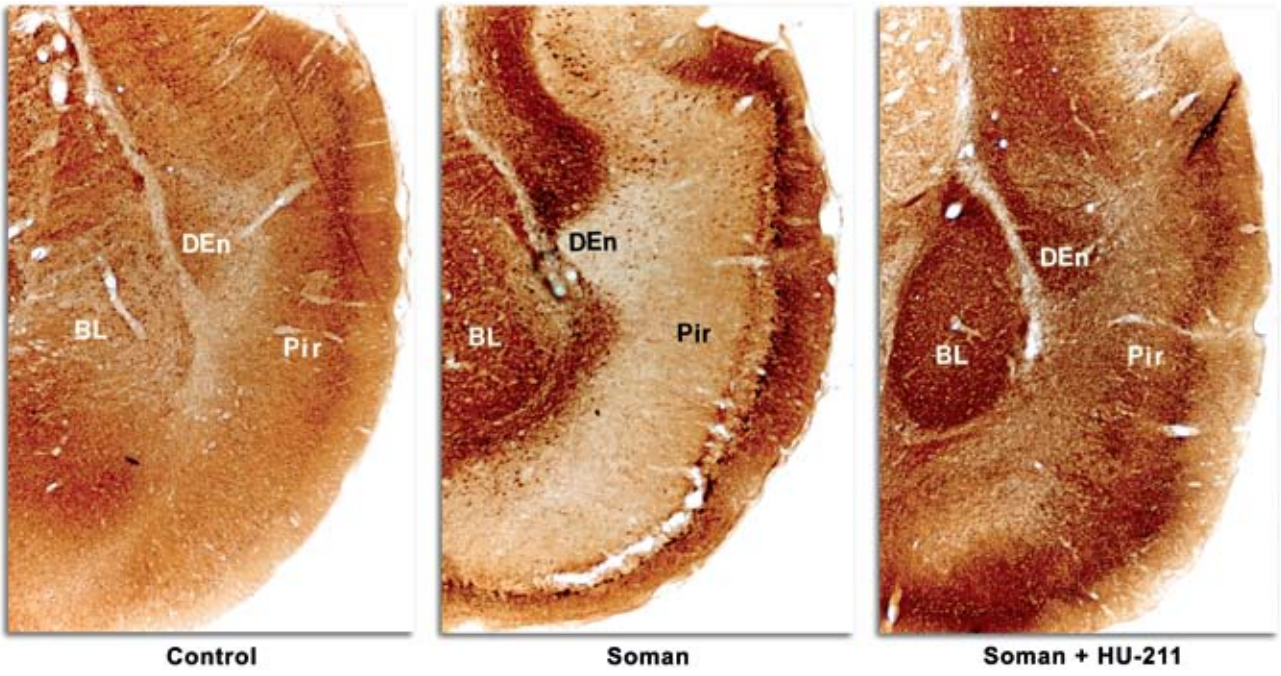

Fig. 6-2. Dexanabinol (HU-211) protects against soman-induced neurological damage. Microtubule-associated protein 2 (MAP-2) staining is neuron-specific. MAP-2 negative immunostaining indicates necrosis, except in areas of white matter.
BL: basolateral amygdaloid nuclear group
DEn: dorsal endopiriform nucleus
Pir: piriform cortex.

and that produced by interfering with delayed calcium overload. In the above studies, HU-211 was protective, despite the continued presence of undiminished seizures and SE, whereas diazepam attenuated (without stopping) seizure intensity and thereby reduced the initial insult. The anticonvulsant action of diazepam, via agonistic modulation of γ-aminobutyric A (GABA[A]) receptors, is well known. These mechanisms are nonoverlapping, and neuroprotective effects should be additive or synergistic. HU-211 is not approved for clinical use, and the company that owns the rights to it (Pharmos Ltd, Israel) is developing it as a possible adjunctive therapeutic for head trauma.

Gacyclidine

Gacyclidine (GK-11) is another NMDA receptor antagonist that has shown considerable neuroprotective efficacy. When GK-11 (0.01–0 .1 mg/kg, IV) was given to rats 10 minutes after soman exposure (in conjunction with PB pretreatment, and AS, 2-PAM, and diazepam posttreatments, 1 min after soman injections), it completely blocked SRBD when assessed 3 weeks after exposure.[136] In a more realistic battlefield scenario, GK-11 was administered 45 minutes after an exposure of 8 times the median lethal dose (LD_{50}) of soman in nonhuman primates. Animals also received PB pretreatment, followed by AS, 2-PAM, and diazepam posttreatments (1 min after soman exposure) equivalent to a single autoinjector of each in humans. When brain pathology was assessed 3 weeks after exposure, all three GK-11–treated primates showed little or no evidence of pathology in the frontal and entorhinal cortices, amygdala, caudate nucleus, hippocampus, thalamus, midbrain, pons, medulla, and cerebellum, compared with the only surviving soman-treated animal (1 of 3) that received AS, 2-PAM, and diazepam but not GK-11.[137] In a study that approximates casualty management following a terrorist attack, soman-intoxicated (2 times the LD_{50}) primates did not receive PB pretreatment and received delayed AS, 2-PAM, and diazepam treatments (one human-equivalent of each, as above) 30 minutes postexposure, followed by GK-11 (0.1 mg/kg, IV). In this study, the addition of GK-11 restored normal EEG activity and completely prevented neuropathology (assessed 5 weeks after exposure), compared with subjects that received AS, 2-PAM, or diazepam alone.[138] GK-11 has a binding affinity for NMDA receptors that is only one tenth that of MK-801. In addition, it binds to non-NMDA receptors when interaction with NMDA receptors is prevented. For these reasons, GK-11 is considered substantially less neurotoxic than MK-801.[139] It is currently being evaluated in human clinical trials for a different neuroprotective indication.[139,140]

Ketamine

Ketamine appears to be the most promising neuroprotectant candidate to date,[141,142] and it should be used in combination with a benzodiazepine, such as diazepam. Ketamine is an FDA-approved anesthetic that blocks neurotransmissions without depressing respiratory and circulatory functions. Its actions are mediated by low-affinity binding to NMDA receptor channels and prevention of calcium influx.[142–145] Ketamine is garnering considerable attention as a putative neuroprotectant against ischemic brain injury, damage resulting from seizures and SE, irrespective of etiology, and SRBD specifically resulting from nerve-agent–induced seizures.[144–149] Fujikawa[147]reported remarkable neuroprotection in 21 of 24 brain regions in rats when 100 mg/kg of ketamine was administered (IP) 15 minutes after lithium-pilocarpine-induced SE onset. Similarly, 100 mg/kg of ketamine (IP) prevented learning impairment in rats when administered immediately after lithium-pilocarpine-induced SE.[150] Borris et al[151] report that ketamine (58 mg/kg, the effective dose in 50% of those taking it [ED_{50}]) can control prolonged SE in rats when administered 1 hour after onset. Cumulative evidence for the beneficial effects of ketamine following SE onset has led to its recommended use in humans when SE cannot be alleviated by conventional anticonvulsant therapy.[148]

Based on its neuroprotective and anticonvulsant properties, Mion et al[145] recommend ketamine for victims of nerve agent exposure. More recently, Dorandeu et al[149] reported that ketamine proved effective in stopping seizures, highly reducing SRBD, and improving guinea pig survival when administered between 30 minutes and 2 hours after soman poisoning. Increasing dosages of ketamine (ie, 10–60 mg/kg, IM) were required as post-SE onset delay increased, and ketamine was always administered with atropine sulfate (2–10 mg/kg); in addition, guinea pigs received pyridostigmine (26 mg/kg, IM) 30 minutes prior to soman and AMN (4 mg/kg, IM) within 1 minute following the soman injection. Their study also provided compelling evidence of neuroprotection by ketamine at dosages that did not modify seizures (ie, 2–10 mg/kg), and suggested combining ketamine and benzodiazepine treatments when treatment is delayed 2 hours.

Results from the authors' laboratory corroborate reports of neuroprotection by ketamine following soman-induced SE. The authors observed that neuroprotection was greatly augmented by administering ketamine plus diazepam, compared to diazepam alone. When soman-exposed (1.6 times the LD_{50}) rats were administered 20 mg/kg diazepam (IM) and 25 mg/kg ketamine (IP), 40 minutes after seizure onset,

the mean cross-sectional area of temporal lobe necrosis (ie, piriform cortex and surrounding structures) was reduced by 85.5% compared to soman-positive controls ($P = 0.018$). The mean reduction produced by diazepam alone was only 39.9% and was not significant. In the lateral dorsal thalamus and surrounding thalamic nuclei, diazepam plus ketamine reduced severe damage by 91.4% compared to soman controls ($P < 0.001$). The reduction in lateral dorsal thalamus damage by diazepam alone was only 27.4% and was not significant. Neuronal pathological assessments, using haematoxylin and eosin stain, confirmed these quantitative findings. It is likely that reduced seizure intensities contributed to the observed neuroprotection; however, this speculation is unconfirmed because EEGs were not obtained from these animals.

Taken together, the preponderance of evidence indicates that ketamine is a viable neuroprotectant candidate against nerve-agent–induced SRBD. However, ketamine is not FDA approved for this purpose. There have been no human or nonhuman primate studies to determine the optimal dose of ketamine to be used in combination with diazepam or other benzodiazepines to alleviate nerve-agent–induced SE. On the other hand, several case reports describe the effectiveness of ketamine, following benzodiazepine therapy, for refractory human SE from different causes. Therefore, off-label use of ketamine, as adjunct neuroprotective therapy following nerve agent intoxication, should be undertaken with caution and consideration of the best available evidence.

Because ketamine would be administered in conjunction with diazepam, and because of an increased risk of respiratory insufficiency by the combined treatments (see below), it is important to review treatment recommendations for diazepam. The autoinjector issued by the US military contains 10 mg diazepam. For a 70-kg (154-lb) individual, one autoinjector delivers a dose (0.14 mg/kg, IM) consistent with the diazepam loading dosage (0.15 mg/kg, IV) recommended by the recent Belgian Consensus on SE.[148] The autoinjector dose is also consistent with the diazepam dose (5–20 mg/70 kg) recommended by Durham[152] as initial treatment for SE, and is in agreement with the 20-mg diazepam dose (per rectum) recommended in "Treatment of Status Epilepticus in Adults: Columbia University Protocol," as first line therapy when IV access is not available.[153] The Belgian Consensus[148] further recommends 4 to 8 mg per hour IV maintenance dosing with diazepam. On the battlefield, medics and unit lifesavers are permitted to administer two additional 10-mg dosages of diazepam. Overall there is regularity in the recommended use of diazepam in the initial treatment of adult SE, regardless of cause. The main adverse effects of diazepam, and benzodiazepines in general, are respiratory depression, hypotension, and decreased consciousness.[148]

For intractable SE, the Belgian Consensus advocates an adult dosage of 50 to 100 mg ketamine as a follow up to diazepam for its "theoretical neuroprotective effects."[148] This dosage is consistent with Durham's[152] recommendation of 50 to 100 mg ketamine followed by 50 to 100 mg per hour, as a "second-line" treatment for refractory SE. Walker et al[154] report successfully treating an adult patient exhibiting "partial motor SE" with an anesthetic dosage of ketamine (ie, 100 mg/h). In a 13-year-old girl whose SE failed to respond to all standard treatments, control of clinical and electrographic SE was obtained within 90 seconds following a bolus injection (IV) of 2 mg/kg ketamine; control was maintained by continuous infusion of ketamine up to a maximum of 7.5 mg/kg per hour.[155]

Adverse effects of ketamine include a transient decrease in respiratory rate with bolus administration (ie, ≥ 2 mg/kg, IV), pulmonary secretions (controllable with atropine), transient cardiovascular stimulation and possible tachycardia, intracranial hypertension (making it contraindicated for closed head injury), and undesired psychic effects.[148,156] In field situations, ketamine is preferred above other anesthetics because it is relatively unlikely to cause respiratory depression. It is generally accepted that ketamine does not produce significant ventilatory depression in humans.[156]

Ketamine may also produce neurotoxicity typical of NMDA receptor antagonists. As mentioned above, NMDA receptor antagonists have been shown to cause neurotoxicity in the cingulate and retrosplenial cortices as well as cerebellar Purkinje cells.[117,118,157,158] A case of possible ketamine toxicity was seen in a 44-year-old man treated for refractory SE.[158] Control of his SE was achieved with an initial bolus injection of 2 mg/kg ketamine (IV, over 2 min), followed by a continuous infusion of 2 mg/kg per hour. Infusion dosages were progressively increased until achieving a final dose of 7.5 mg/kg per hour after 48 hours. Dosages were then titrated down over the next 72 hours. The patient exhibited diffuse cerebellar and cerebral atrophy consistent with animal models of NMDA antagonist-mediated neurotoxicity.[158] Studies have reported that the mechanism of this toxicity is indirectly mediated by excessive cholinergic stimulation,[119–121] and supplemental atropine could have an ameliorative effect. In addition, GABAergic stimulation is reportedly protective against this specific form of neurotoxicity.[119–121]

However, high dosages of both diazepam and ketamine could exacerbate respiratory distress already present in nerve agent casualties. Therefore, a conservative dose range for ketamine is advisable. In humans, a ketamine dose less than 1 mg/kg, IV, provides effective analgesia against acute and chronic pain.[146,156,159]

The anesthetic dose range in humans is 5 to 10 mg/kg, IV.[146,159] For a nerve agent victim on the battlefield, a ketamine dosage below 2 mg/kg, IV, should prove safe in combination with the high dosages of diazepam that are likely to be administered. While possibly not high enough to augment the anticonvulsant effects of diazepam and arrest SE, anesthetic or subanesthetic dosages of ketamine should provide considerable additional neuroprotection, compared to diazepam alone. Moreover, the ketamine dosage can be increased once patients reach a medical facility where intubation and ventilation can be provided.

ADDITIONAL NEUROPROTECTIVE APPROACHES

Free Radical Scavengers

Damage produced by reactive oxygen species or free radicals is a component of seizure and SE-related neurotoxicity,[47,160,161] including damage resulting from nerve agent poisoning.[160] The liberation of catalytic iron from extravasated hemoglobin may generate reactive oxygen species.[160,161] Reactive oxygen species could also be generated by xanthine oxidase or impaired mitochondrial electron transport,[161–163] offering the hope that nerve-agent–induced neurotoxicity could be mitigated by antioxidants or free radical scavengers.

Nitrone-based free radical traps, such as alpha-phenyl-N-tert-butylnitrone (PBN), which react with reactive oxygen species, have proven to be neuroprotective following cholinesterase inhibition. Pretreatment with PBN prevented seizures induced by diisofluorophosphate, an organophosphonate and nerve agent simulant.[164] Moreover, PBN (150 mg/kg, IP, 5 min after seizure onset) produced significant neuroprotection in the piriform cortices and other cortical areas of rats following lithium pilocarpine-induced SE.[165] Unfortunately (and reminiscent of the findings with HU-211 discussed above), thalamic damage was either exacerbated or not diminished by PBN in the latter study. Another report describes neuroprotective effects by PBN 12 hours after ischemic insult.[166] A pilot study of PBN did not show neuroprotection against soman-induced injury.[167] A new, centrally acting, nitrone-based free radical scavenger, S34176, has shown superior neuroprotective properties compared to PBN in stroke and other glutamate excitotoxicity models.[168] S34176 may prove useful against nerve-agent–induced injury.

Mitochondrial Permeability Transition Inhibitors

As mentioned above, damaging stimuli can induce neuronal mitochondria to undergo permeability transition, forming pores that allow the release of stored calcium into the neuronal cytoplasm. This is accompanied by curtailment of ATP synthesis, mitochondrial swelling, exacerbation of calcium overload, and neuronal death.[59–62] The assembly of mitochondrial transition pores can be blocked by cyclosporin A, an FDA -approved drug used in cancer chemotherapy. There is evidence that cyclosporin A and topiramate (another transition pore blocker) are neuroprotective in various models of excitotoxic brain injury.[169–174] Bauman and colleagues[169] found that cyclosporin A dramatically reduced brain injury in rats following seizures and SE induced by the organophosphate paraoxon. There is also evidence of neuroprotection by topiramate following pilocarpine-induced seizures and SE.[170]

Neuroprotective Hypothermia

Total-body cooling is an effective nonpharmacologic method of treating cerebrovascular disease. Several stroke experts have advanced this approach as holding great promise in reducing the amount of ischemic brain damage, and in 2004 the FDA approved a catheter for stroke and other specific uses that cools the blood in a penetrating artery. Less technologically complicated approaches to total-body cooling have been successful in limited numbers of animal studies.[175,176] Whether this approach would be practical in a battlefield situation, especially with mass casualties, is questionable, but it should be kept in mind as a possibility.

SUMMARY

A variety of neuroprotective compounds have proven useful in alleviating brain damage caused by nerve-agent–induced seizures and SE. Of these, ketamine, memantine, and dantrolene have received FDA approval for other indications, and several other compounds are in clinical trials. Based on the evidence, ketamine, in combination with diazepam, is the top candidate and most viable neuroprotectant for nerve agent survivors exhibiting seizures and SE. A dantrolene and diazepam combination is a viable possibility as well, though less efficacious. In addition, free radical scavengers (eg, S34176) and transition pore blockers (eg, cyclosporin A) show great promise. It is conceivable that the best possible neuroprotective approach will be a "cocktail" of two or more agents that affect, in a synergistic fashion, different legs of the excitotoxic pathway.[177]

REFERENCES

1. Solberg Y, Belkin M. The role of excitotoxicity in organophosphorous nerve agents central poisoning. *Trends Pharmacol Sci*. 1997;18:183–185.

2. Shih TM, Duniho SM, McDonough JH. Control of nerve agent-induced seizures is critical for neuroprotection and survival. *Toxicol Appl Pharm*. 2003;188:69–80.

3. DeLorenzo RJ. Management of status epilepticus. *Va Med Q*. 1996;123:103–111.

4. DeLorenzo RJ, Hauser WA, Towne AR, et al. A prospective, population-based epidemiologic study of status epilepticus in Richmond, Virginia. *Neurology*. 1996;46:1029–1035.

5. Newmark J. The birth of nerve agent warfare: lessons from Syed Abbas Foroutan. *Neurology*. 2004;62:1590–1596.

6. Sidell FR. Nerve Agents. In: Sidell FR, Takafuji ET, Franz DR, eds. *Medical Aspects of Chemical and Biological Warfare*. In: Zajtchuk R, Bellamy RF, eds. *Textbook of Military Medicine*. Washington, DC: Department of the Army, Office of The Surgeon General, Borden Institute; 1997: Chap 5.

7. Lipp JA. Effect of diazepam upon soman-induced seizure activity and convulsions. *Electroencephalogr Clin Neurophysiol*. 1972;32:557–560.

8. Lipp JA. Effect of benzodiazepine derivatives on soman-induced seizure activity and convulsions in the monkey. *Arch Int Pharmacodyn Ther*. 1973;202:244–251.

9. McDonough JH Jr, Jaax NK, Crowley RA, Mays MZ, Modrow HE. Atropine and/or diazepam therapy protects against soman-induced neural and cardiac pathology. *Fundam Appl Toxicol*. 1989;13:256–276.

10. McDonough JH Jr, Shih TM. Pharmacological modulation of soman-induced seizures. *Neurosci Biobehav Rev*. 1993;17:203–215.

11. Shih TM. Anticonvulsant effects of diazepam and MK-801 in soman poisoning. *Epilepsy Res*. 1990;7:105–116.

12. Shih TM, Koviak TA, Capacio BR. Anticonvulsants for poisoning by the organophosphorus compound soman: pharmacological mechanisms. *Neurosci Biobehav Rev*. 1991;15:349–362.

13. Capacio BR, Shih TM. Anticonvulsant actions of anticholinergic drugs in soman poisoning. *Epilepsia*. 1991;32:604–615.

14. Philippens IH, Melchers BP, de Groot DM, Wolthuis OL. Behavioral performance, brain histology, and EEG sequela after immediate combined atropine/diazepam treatment of soman-intoxicated rats. *Pharmacol Biochem Behav*. 1992;42:711–719.

15. Sparenborg S, Brennecke LH, Beers ET. Pharmacological dissociation of the motor and electrical aspects of convulsive status epilepticus induced by the cholinesterase inhibitor soman. *Epilepsy Res*. 1993;14:95–103.

16. Harris LW, Gennings C, Carter WH, Anderson DR, Lennox WJ, Bowersox SL, Solana RP. Efficacy comparison of scopolamine (SCP) and diazepam (DZ) against soman-induced lethality in guinea pigs. *Drug Chem Toxicol*. 1994;17:35–50.

17. Shih T, McDonough JH Jr, Koplovitz I. Anticonvulsants for soman-induced seizure activity. *J Biomed Sci*. 1999;6:86–96.

18. Lallement G, Renault F, Baubichon D, et al. Compared efficacy of diazepam or avizafone to prevent soman-induced electroencephalographic disturbances and neuropathology in primates: relationship to plasmatic benzodiazepine pharmacokinetics. *Arch Toxicol*. 2000;74:480–486.

19. McDonough JH Jr, Zoeffel LD, McMonagle J, Copeland TL, Smith CD, Shih TM. Anticonvulsant treatment of nerve agent seizures: anticholinergic versus diazepam in soman-intoxicated guinea pigs. *Epilepsy Res*. 2000;38:1–14.

20. Clement JG, Broxup B. Efficacy of diazepam and avizafone against soman-induced neuropathology in brain of rats. *Neurotoxicology*. 1993;14:485–504.

21. Hayward IJ, Wall HG, Jaax NK, Wade JV, Marlow DD, Nold JB. Decreased brain pathology in organophosphate-exposed rhesus monkeys following benzodiazepine therapy. *J Neurol Sci*. 1990;98:99–106.

22. McDonough JH Jr, Dochterman LW, Smith CD, Shih TM. Protection against nerve agent-induced neuropathology, but not cardiac pathology, is associated with the anticonvulsant action of drug treatment. *Neurotoxicology*. 1995;16:123–132.

23. Baze WB. Soman-induced morphological changes: an overview in the non-human primate. *J Appl Toxicol*. 1993;13:173–177.

24. Olney JW, de Gubareff T, Labruyere J. Seizure-related brain damage induced by cholinergic agents. *Nature*. 1983;301:520–522.

25. Sloviter RS. "Epileptic" brain damage in rats induced by sustained electrical stimulation of the perforant path. I. Acute electrophysiological and light microscopic studies. *Brain Res Bull*. 1983;10:675–697.

26. Meldrum BS. Concept of activity-induced cell death in epilepsy: historical and contemporary perspectives. *Progress Brain Res*. 2002;135:3–11.

27. Fanzone JF, Levine ES, Hursh SR. *Nerve Agent Bioscavenger Pretreatment Against Chemical Warfare Agents: Challenge, Casualty, and Intervention Modeling Support*. Aberdeen Proving Ground, Md: US Army Medical Research and Materiel Command. Interim Report. Contract No. DAMD17-98-D-0022; 2002.

28. DeLorenzo RJ, Waterhouse EJ, Towne AR, et al. Persistent nonconvulsive status epilepticus after the control of convulsive status epilepticus. *Epilepsia*. 1998;39:833–840.

29. Petras JM. Soman neurotoxicity. *Fundam Appl Toxicol*. 1981;1:242.

30. Lemercier G, Carpentier P, Sentenac-Roumanou H, Morelis P. Histological and histochemical changes in the central nervous system of the rat poisoned by an irreversible anticholinesterase organophosphorus compound. *Acta Neuropathol (Berl)*. 1983;61:123–129.

31. McLeod CG Jr, Singer AW, Harrington DG. Acute neuropathology in soman poisoned rats. *Neurotoxicology*. 1984;5:53–57.

32. Carpentier P, Delamanche IS, Le Bert M, Blanchet G, Bouchaud C. Seizure-related opening of the blood-brain barrier induced by soman: possible correlation with the acute neuropathology observed in poisoned rats. *Neurotoxicology*. 1990;11:493–508.

33. Kadar T, Cohen G, Sahar R, Alkalai D, Shapira S. Long-term study of brain lesions following soman, in comparison to DFP and metrazol poisoning. *Hum Exp Toxicol*. 1992; 11:517–523.

34. McDonough JH Jr, McLeod CG Jr, Nipwoda MT. Direct microinjection of soman or VX into the amygdala produces repetitive limbic convulsions and neuropathology. *Brain Res*. 1987;435:123–137.

35. Pazdernik TL, Cross R, Giesler M, Nelson S, Samson F, McDonough J Jr. Delayed effects of soman: brain glucose use and pathology. *Neurotoxicology*. 1985;6:61–70.

36. Petrali JP, Maxwell DM, Lenz DE, Mills KR. Effect of an anticholinesterase compound on the ultrastructure and function of the rat blood-brain barrier: a review and experiment. *J Submicrosc Cytol Pathol*. 1991;23:331–338.

37. Petras JM. Neurology and neuropathology of soman-induced brain injury: an overview. *J Exp Anal Behav*. 1994;61:319–329.

38. Ballough GP, Martin LJ, Cann FJ, et al. Microtubule-associated protein 2 (MAP-2): a sensitive marker of seizure-related brain damage. *J Neurosci Methods*. 1995;61:23–32.

39. Lallement G, Carpentier P, Collet A, Baubichon D, Pernot-Marino I, Blanchet G. Extracellular acetylcholine changes in rat limbic structures during soman-induced seizures. *Neurotoxicology*. 1992;13:557–567.

40. Tonduli LS, Testylier G, Marino IP, Lallement G. Triggering of soman-induced seizures in rats: multiparametric analysis with special correlation between enzymatic, neurochemical, and electrophysiological data. *J Neurosci Res*. 1999;58:464–473.

41. Wade JV, Samson FE, Nelson SR, Pazdernik TL. Changes in extracellular amino acids during soman- and kainic acid-induced seizures. *J Neurochem*. 1987;49:645–650.

42. Braitman DJ, Sparenborg S. MK-801 protects against seizures induced by the cholinesterase inhibitor soman. *Brain Res Bull*. 1989;23:145–148.

43. Sparenborg S, Brennecke LH, Jaax NK, Braitman DJ. Dizocilpine (MK-801) arrests status epilepticus and prevents brain damage induced by soman. *Neuropharmacology*. 1992;31:357–368.

44. Fosbraey P, Wetherell JR, French MC. Neurotransmitter changes in guinea-pig brain regions following soman intoxication. *J Neurochem*. 1990;54:72–79.

45. Choi DW. Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. *Trends Neurosci*. 1988;11:465–469.

46. Shih TM, Capacio BR, Cook LA. Effects of anticholinergic-antiparkinsonian drugs on striatal neurotransmitter levels of rats intoxicated with soman. *Pharmacol Biochem Behav*. 1993;44:615–622.

47. Fujikawa DG. Prolonged seizures and cellular injury: understanding the connection. *Epilepsy Behav*. 2005:7(suppl 3): S3–11.

48. Carafoli E. Calcium signaling: a tale for all seasons. *Proc Natl Acad Sci U S A*. 2002;99:1115–1122.

49. Verkhratsky A, Toescu EC. Endoplasmic reticulum Ca(2+) homeostasis and neuronal death. *J Cell Mol Med*. 2003;7:351–361.

50. Parekh AB. Store-operated Ca2+ entry: dynamic interplay between endoplasmic reticulum, mitochondria and plasma membrane. *J Physiol*. 2003;547(pt 2):333–348.

51. Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*. 1993;361:31–39.

52. Randall RD, Thayer SA. Glutamate-induced calcium transient triggers delayed calcium overload and neurotoxicity in rat hippocampal neurons. *J Neurosci*.1992;12:1882–1895.

53. Orrenius S, Burkitt MJ, Kass GE, Dypbukt JM, Nicotera P. Calcium ions and oxidative cell injury. *Ann Neurol*. 1992; 32(suppl):S33–42.

54. Orrenius S, Nicotera P. The calcium ion and cell death. *J Neural Transm Suppl*. 1994;43:1–11.

55. Nicotera P. Molecular switches deciding the death of injured neurons. *Toxicol Sci*. 2003;74:4–9.

56. Nicholls DG. Mitochondrial dysfunction and glutamate excitotoxicity studied in primary neuronal cultures. *Curr Mol Med*. 2004;4:149–177.

57. Jayakar SS, Dikshit M. AMPA receptor regulation mechanisms: future target for safer neuroprotective drugs. *Int J Neurosci*. 2004;114:695–734.

58. Kulak W, Sobaniec W, Wojtal K, Czuczwar SJ. Calcium modulation in epilepsy. *Pol J Pharmacol*. 2004;56:29–41.

59. Duchen MR. Mitochondria and calcium: from cell signaling to cell death. *J Physiology*. 2000;529:57–68.

60. Halestrap AP, McStay GP, Clarke SJ. The permeability transition pore complex: another view. *Biochimie*. 2002;84:153–166.

61. Chang LK, Putcha GV, Deshmukh M, Johnson EM Jr. Mitochondrial involvement in the point of no return in neuronal apoptosis. *Biochimie*. 2002;84:223–231.

62. Mattson MP. Excitotoxic and excitoprotective mechanisms: abundant targets for the prevention and treatment of neurodegenerative disorders. *Neuromolecular Med*. 2003;3:65–94.

63. Hou ST, MacManus JP. Molecular mechanisms of cerebral ischemia-induced neuronal death. *Intern Rev Cytol*. 2002;221:93–149.

64. Clarke PGH. Apoptosis versus necrosis--how valid a dichotomy for neurons: In: Koliatsos VE, Ratan RR, eds. *Cell Death and Diseases of the Nervous System*. Totowa, NJ: Humana Press Inc; 1999: 3–28.

65. Sloviter RS. Apoptosis: a guide for the perplexed. *Trends Pharmacol Sci* 2002;23:19–24.

66. Martin LJ, Al-Abdulla NA, Brambrink AM, Kirsch JR, Sieber FE, Portera-Cailliau C. Neurodegeneration in excitotoxicity, global cerebral ischemia, and target deprivation: a perspective on the contributions of apoptosis and necrosis. *Brain Res Bull*. 1998;46:281–309.

67. Baille V, Clarke PG, Brochier G, et al. Soman-induced convulsions: the neuropathology revisited. *Toxicology*. 2005;215:1–24.

68. Ballough GP, Forster JS, Makowski JP, Sordoni NC, Filbert MG. Soman-induced seizures produce neuronal apoptosis [abstract]. *Abstr Soc Neurosci*. 1997;23:1936.

69. Leist M, Single B, Castoldi AF, Kühnle S, Nicotera P. Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *J Exp Med*. 1997;185:1481–1486.

70. Nicotera P, Leist M, Ferrando-May E. Intracellular ATP, a switch in the decision between apoptosis and necrosis. *Toxicol Lett*. 1998;102–103:139–142.

71. Shoulson I. Experimental therapeutics of neurodegenerative disorders: unmet needs. *Science*. 1998;282:1072–1074.

72. Schulz, JB. Neurodegeneration. In: Bahr M, ed. *Neuroprotection: Models, Mechanisms, and Therapies*. Weinheim, Baden Württemberg, Germany: Wiley-VCH Verlag GmbH & Co; 2004: Chap 16.

73. Siesjo BK, Bengtsson F. Calcium fluxes, calcium antagonists, and calcium-related pathology in brain ischemia, hypoglycemia, and spreading depression: a unifying hypothesis. *J Cereb Blood Flow Metab*. 1989;9:127–140.

74. Silver B, Weber J, Fisher M. Medical therapy for ischemic stroke. *Clin Neuropharmacol*. 1996;19:101–128.

75. Costa E, Armstrong DM, Guidotti A, et al. Gangliosides in the protection against glutamate excitotoxicity. *Prog Brain Res*. 1994;101:357–373.

76. Washington University. The Internet Stroke Center. Available at: www.strokecenter.org. Accessed March 21, 2007.

77. Orbach J. *The Neuropsychological Theories of Lashley and Hebb: Contemporary Perspectives Fifty Years After Hebb's* The Organization of Behavior, *Vanuxem Lectures and Selected Theoretical Papers of Lashley*. Lanham, Md: University Press of America; 1998.

78. Choi DW. Ionic dependence of glutamate neurotoxicity. *J Neurosci*. 1987;7:369–379.

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

80. Shih TM, McDonough JH Jr. Neurochemical mechanisms in soman-induced seizures. *J Appl Toxicol*. 1997;17:255–264.

81. McDonough JH Jr, Shih TM. Neuropharmacological mechanisms of nerve agent-induced seizure and neuropathology. *Neurosci Biobehav Rev.* 1997;21:559–579.

82. Manev H, Guidotti A, Costa E. Protection by gangliosides against glutamate excitotoxicity. *Adv Lipid Res*. 1993;25:269–288.

83. Tubaro E, Santiangeli C, Cavallo G, et al. Effect of a new de-N-acetyl-lysoglycosphingolipid on chemically-induced inflammatory bowel disease: possible mechanism of action. *Naunyn Schmiedebergs Arch Pharmacol.* 1993;348:670–678.

84. Otani S, Daniel H, Takita M, Crepel F. Long-term depression induced by postsynaptic group II metabotropic glutamate receptors linked to phospholipase C and intracellular calcium rises in rat prefrontal cortex. *J Neurosci*. 2002;22:3434–3444.

85. Monnet FP, Morin-Surun MP, Leger J, Combettes L. Protein kinase C-dependent potentiation of intracellular calcium influx by sigma1 receptor agonists in rat hippocampal neurons. *J Pharmacol Exp Ther*. 2003;307:705–312.

86. Chaban VV, Li J, Ennes HS, Nie J, Mayer EA, McRoberts JA. N-methyl-D-aspartate receptors enhance mechanical responses and voltage-dependent Ca2+ channels in rat dorsal root ganglia neurons through protein kinase C. *Neuroscience*. 2004;128:347–357.

87. Niebauer M, Gruenthal M. Neuroprotective effects of early vs. late administration of dantrolene in experimental status epilepticus. *Neuropharmacology*. 1999;38:1343–1348.

88. Ballough GPH, Filbert MG. *A Viable Neuroprotection Strategy Following Soman-Induced Status Epilepticus*. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 2003. USAMRICD Technical Report 03-09, AD A443565.

89. Krause T, Gerbershagen MU, Fiege M, Weisshorn R, Wappler F. Dantrolene–a review of its pharmacology, therapeutic use, and new developments. *Anaesthesia*. 2004;59:364–373.

90. Ballough GP, Cann FJ, Smith CD, Forster JS, Kling CE, Filbert MG. GM1 monosialoganglioside pretreatment protects against soman-induced seizure-related brain damage. *Mol Chem Neuropathol*. 1998;34:1–23.

91. Ando S. Gangliosides in the nervous system. *Neurochem Int*. 1983;5:507–537.

92. Ledeen RW. Biology of gangliosides: neuritogenic and neuronotrophic properties. *J Neurosci Res*. 1984;12:147–159.

93. Yu RK, Goldenring JR, Kim JYH, DeLorenzo RJ. Gangliosides as differential modulators of membrane-bound protein kinase systems. In: Tettamanti G, Ledeen RW, Sandhoff K, Nagai Y, Toffano G, eds. *Fidia Research Series: Gangliosides and Neuronal Plasticity*. Vol 6. Padova, Italy: Liviana Press; 1986: 95–104.

94. Vaccarino F, Guidotti A, Costa E. Ganglioside inhibition of glutamate-mediated protein kinase C translocation in primary cultures of cerebellar neurons. *Proc Natl Acad Sci U S A*. 1987;84:8707–8711.

95. Manev H, Favaron M, Vicini S, Guidotti A. Ganglioside-mediated protection from glutamate-induced neuronal death. *Acta Neurobiol Exp (Wars).* 1990;50:475–488.

96. Wagey R, Hu J, Pelech SL, Raymond LA, Krieger C. Modulation of NMDA-mediated excitotoxicity by protein kinase C. *J Neurochem*. 2001;78:715–726.

97. Koponen S, Kurkinen K, Akerman KE, Mochly-Rosen D, Chan PH, Koistinaho J. Prevention of NMDA-induced death of cortical neurons by inhibition of protein kinase Czeta. *J Neurochem*. 2003;86:442–450.

98. Zhang L, Rzigalinski BA, Ellis EF, Satin LS. Reduction of voltage-dependent Mg2+ blockade of NMDA current in mechanically injured neurons. *Science*. 1996;274:1921–1923.

99. Tubaro E, Croce C, Cavallo G, Belogi L, Guida G, Santiangeli C, Cifone MG, Santoni A, Mainiero F. In vitro and in vivo impact of a new glycosphingolipid on neutrophils. *Agents Actions*. 1994;42:107–113.

100. Mattei V, Garofalo T, Misasi R, Gizzi C, Mascellino MT, Dolo V, Pontieri GM, Sorice M, Pavan A. Association of cellular prion protein with gangliosides in plasma membrane microdomains of neural and lymphocytic cells. *Neurochem Res*. 2002;27:743–749.

101. Eliasson MJ, Sampei K, Mandir AS, et al. Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. *Nat Med*. 1997;3:1089–1095.

102. Mandir AS, Poitras MF, Berliner AR, et al. NMDA but not non-NMDA excitotoxicity is mediated by Poly(ADP-ribose) polymerase. *J Neurosci*. 2000;20:8005–8011.

103. Whalen MJ, Clark RS, Dixon CE, et al. Traumatic brain injury in mice deficient in poly-ADP(ribose) polymerase: a preliminary report. *Acta Neurochir Suppl*. 2000;76:61–64.

104. Abdelkarim GE, Gertz K, Harms C, et al. Protective effects of PJ34, a novel, potent inhibitor of poly(ADP-ribose) polymerase (PARP) in in vitro and in vivo models of stroke. *Int J Mol Med*. 2001;7:255–260.

105. Kamanaka Y, Kondo K, Ikeda Y, et al. Neuroprotective effects of ONO-1924H, an inhibitor of poly ADP-ribose polymerase (PARP), on cytotoxicity of PC12 cells and ischemic cerebral damage. *Life Sci*. 2004;76:151–162.

106. Sharma SS, Munusamy S, Thiyagarajan M, Kaul CL. Neuroprotective effect of peroxynitrite decomposition catalyst and poly(adenosine diphosphate-ribose) polymerase inhibitor alone and in combination in rats with focal cerebral ischemia. *J Neurosurg*. 2004;101:669–675.

107. Nakajima H, Kakui N, Ohkuma K, Ishikawa M, Hasegawa T. A newly synthesized poly(ADP-ribose) polymerase inhibitor, DR2313 [2-methyl-3,5,7,8-tetrahydrothiopyrano[4,3-d]-pyrimidine-4-one]: pharmacological profiles, neuroprotective effects, and therapeutic time window in cerebral ischemia in rats. *J Pharmacol Exp Ther*. 2005;312:472–481.

108. Ying W, Alano CC, Garnier P, Swanson RA. NAD+ as a metabolic link between DNA damage and cell death. *J Neurosci Res*. 2005;79:216–223.

109. Szabo C, Dawson VL. Role of poly(ADP-ribose) synthetase in inflammation and ischaemia-reperfusion. *Trends Pharmacol Sci*. 1998;19:287–298.

110. Virag L, Szabo C. The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. *Pharmacol Rev*. 2002;54:375–429.

111. Park EM, Cho S, Frys K, et al. Interaction between inducible nitric oxide synthase and poly(ADP-ribose) polymerase in focal ischemic brain injury. *Stroke*. 2004;35:2896–2901.

112. Wang H, Yu SW, Koh DW, et al. Apoptosis-inducing factor substitutes for caspase executioners in NMDA-triggered excitotoxic neuronal death. *J Neurosci*. 2004;24:10963–10973.

113. Meier HL, Ballough GP, Forster JS, Filbert MG. Benzamide, a poly(ADP-ribose) polymerase inhibitor, is neuroprotective against soman-induced seizure-related brain damage. *Ann N Y Acad Sci*. 1999;890:330–335.

114. Iwashita A, Tojo N, Matsuura S, et al. A novel and potent poly(ADP-ribose) polymerase-1 inhibitor, FR247304 (5-chloro-2-[3-(4-phenyl-3,6-dihydro-1(2H)-pyridinyl)propyl]-4(3H)-quinazolinone), attenuates neuronal damage in vitro and in vivo models of cerebral ischemia. *J Pharmacol Exp Ther*. 2004;310:425–436.

115. Frandsen A, Schousboe A. Dantrolene prevents glutamate cytotoxicity and Ca2+ release from intracellular stores. *J Neurochem*. 1991;56:1075–1078.

116. Popescu BO, Oprica M, Sajin M, et al. Dantrolene protects neurons against kainic acid induced apoptosis in vitro and in vivo. *J Cell Mol Med*. 2002;6:555–569.

117. Olney JW, Labruyere J, Price MT. Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. *Science*. 1989;244:1360–1362.

118. Fix AS, Horn JW, Wightman KA, et al. Neuronal vacuolization and necrosis induced by the noncompetitive N-methyl-D-aspartate (NMDA) antagonist MK(+)801 (dizocilpine maleate): a light and electron microscopic evaluation of the rat retrosplenial cortex. *Exp Neurol*. 1993;123:204–215.

119. Corso TD, Sesma MA, Tenkova TI, et al. Multifocal brain damage induced by phencyclidine is augmented by pilocarpine. *Brain Res*. 1997;752:1–14.

120. Olney JW, Labruyere J, Wang G, Wozniak DF, Price MT, Sesma MA. NMDA antagonist neurotoxicity: mechanism and prevention. *Science*. 1991;254:1515–1518.

121. Wozniak DF, Dikranian K, Ishimaru MJ, et al. Disseminated corticolimbic neuronal degeneration induced in rat brain by MK-801: potential relevance to Alzheimer's disease. *Neurobiol Dis*. 1998;5:305–322.

122. Bormann J. Memantine is a potent blocker of N-methyl-D-aspartate (NMDA) receptor channels. *Eur J Pharmacol*. 1989;166:591–592.

123. Chen HS, Pellegrini JW, Aggarwal SK, et al. Open-channel block of N-methyl-D-aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity. *J Neurosci*. 1992;12:4427–4436.

124. Lipton SA. Excitotoxicity. In: Bahr M, ed. *Neuroprotection: Models, Mechanisms, and Therapies*. Weinheim, Baden Württemberg, Germany: Wiley-VCH Verlag GmbH & Co; 2004: Chap 14.

125. McLean MJ, Gupta RC, Dettbarn WD, Wamil AW. Prophylactic and therapeutic efficacy of memantine against seizures produced by soman in the rat. *Toxicol Appl Pharmacol*. 1992;112:95–103.

126. Koplovitz I, Schulz S, Shutz M, Railer R, Smith F, Okerberg C, Filbert M. Memantine effects on soman-induced seizures and seizure-related brain damage. *Toxicol Meth* 1997;7:227–239.[AQ2]

127. Parsons CG, Danysz W, Quack G. Memantine is a clinically well tolerated N-methyl-D-aspartate (NMDA) receptor antagonist--a review of preclinical data. *Neuropharmacology*. 1999;38:735–767.

128. Chen HS, Wang YF, Rayudu PV, et al. Neuroprotective concentrations of the N-methyl-D-aspartate open-channel blocker memantine are effective without cytoplasmic vacuolation following post-ischemic administration and do not block maze learning or long-term potentiation. *Neuroscience*. 1998;86:1121–1132.

129. Filbert MG, Forster JS, Smith CD, Ballough GP. Neuroprotective effects of HU-211 on brain damage resulting from soman-induced seizures. *Ann N Y Acad Sci*. 1999;890:505–514.

130. Shohami E, Novikov M, Mechoulam R. A nonpsychotropic cannabinoid, HU-211, has cerebroprotective effects after closed head injury in the rat. *J Neurotrauma*. 1993;10:109–119.

131. Biegon A, Joseph AB. Development of HU-211 as a neuroprotectant for ischemic brain damage. *Neurol Res*. 1995;17:275–280.

132. Lavie G, Teichner A, Shohami E, Ovadia H, Leker RR. Long term cerebroprotective effects of dexanabinol in a model of focal cerebral ischemia. *Brain Res*. 2001;901:195–201.

133. Darlington CL. Dexanabinol: a novel cannabinoid with neuroprotective properties. *IDrugs*. 2003;6:976–979.

134. Kitagawa K, Matsumoto M, Niinobe M, et al. Microtubule-associated protein 2 as a sensitive marker for cerebral ischemic damage—immunohistochemical investigation of dendritic damage. *Neurosci* 1989;31:401–411.

135. Gilland E, Bona E, Hagberg H. Temporal changes of regional glucose use, blood flow and microtubule-associated protein 2 immunostaining after hypoxia ischemia in the immature rat brain. *J Cereb Blood Flow Metab*. 1998:18:222–228.

136. Lallement G, Mestries JC, Privat A, et al. GK 11: promising additional neuroprotective therapy for organophosphate poisoning. *Neurotoxicology*. 1997;18:851–856.

137. Lallement G, Clarencon D, Masqueliez C, et al. Nerve agent poisoning in primates: antilethal, anti-epileptic, and neuroprotective effects of GK-11. *Arch Toxicol*. 1998;72:84–92.

138. Lallement G, Clarencon D, Galonnier M, Baubichon D, Burckhart MF, Peoc'h M. Acute soman poisoning in primates neither pretreated nor receiving immediate therapy: value of gacyclidine (GK-11) in delayed medical support. *Arch Toxicol*. 1999;73:115–122.

139. Hirbec H, Gaviria M, Vignon J. Gacyclidine: a new neuroprotective agent acting at the N-methyl-D-aspartate receptor. *CNS Drug Rev*. 2001;7:172–198.

140. Lepeintre JF, D'Arbigny P, Mathe JF, et al. Neuroprotective effect of gacyclidine. A multicenter double-blind pilot trial in patients with acute traumatic brain injury. *Neurochirurgie*. 2004;50(Part 1):83–95.

141. Lallement G. Centre de Recherches du Service de Sante des Armees, La Tronche, France. Personal communication with Ballough G, June 2005.

142. Dorandeu F. Centre de Recherches du Service de Sante des Armees, La Tronche, France. Personal communication with Ballough G, August 2006.

143. MacDonald JF, Nowak LM. Mechanisms of blockade of excitatory amino acid receptor channels. *Trends Pharmacol Sci*. 1990;11:167–172.

144. Werner C, Reeker W, Engelhard K, Lu H, Kochs E. Ketamine racemate and S-(+)-ketamine. Cerebrovascular effects and neuroprotection following focal ischemia. *Anaesthesist*. 1997;46(supp; 1):S55–S60.

145. Mion G, Tourtier JP, Petitjeans F, Dorandeu F, Lallement G, Ruttimann M. Neuroprotective and antiepileptic activities of ketamine in nerve agent poisoning. *Anesthesiology*. 2003;98:1517.

146. Ivani G, Vercellino C, Tonetti F. Ketamine: a new look to an old drug. *Minerva Anestesiol*. 2003;69:468–471.

147. Fujikawa DG. Neuroprotective effect of ketamine administration after status epilepticus onset. *Epilepsia*. 1995;36:186–195.

148. Van Rijckevorsel K, Boon P, Hauman H, et al. Standards of care for adults with convulsive status epilepticus: Belgian consensus recommendations. *Acta Neurol Belg*. 2005;105:111–118.

149. Dorandeu F, Carpentier P, Baubichon D, et al. Efficacy of the ketamine-atropine combination in the delayed treatment of soman-induced status epilepticus. *Brain Res*. 2005;1051:164–175.

150. Stewart LS, Persinger MA. Ketamine prevents learning impairment when administered immediately after status epilepticus onset. *Epilepsy Behav.* 2001;2:585–591.

151. Borris DJ, Bertram EH, Kapur J. Ketamine controls prolonged status epilepticus. *Epilepsy Res.* 2000;42:117–122.

152. Durham D. Management of status epilepticus. *Crit Care Resusc*. 1999;1:344–353.

153. Robakis TK, Hirsch LJ. Literature review, case report, and expert discussion of prolonged refractory status epilepticus. *Neurocrit Care*. 2006;4:35–46.

154. Walker MC, Howard RS, Smith SJ, Miller DH, Shorvon SD, Hirsch NP. Diagnosis and treatment of status epilepticus on a neurological intensive care unit. *QJM*. 1996;89:913–920.

155. Sheth RD, Gidal BE. Refractory status epilepticus: response to ketamine. *Neurology*. 1998;51:1765–1766.

156. Annetta MG, Iemma D, Garisto C, Tafani C, Proietti R. Ketamine: new indications for an old drug. *Curr Drug Targets*. 2005;6:789–794.

157. Nakki R, Koistinaho J, Sharp FR, Sagar SM. Cerebellar toxicity of phencyclidine. *J Neurosci*. 1995;15(pt 2):2097–2108.

158. Ubogu EE, Sagar SM, Lerner AJ, Maddux BN, Suarez JI, Werz MA. Ketamine for refractory status epilepticus: a case of possible ketamine-induced neurotoxicity. *Epilepsy Behav*. 2003;4:70–75.

159. Wolff K, Winstock AR. Ketamine: from medicine to misuse. *CNS Drugs*. 2006;20:199–218.

160. Pazdernik TL, Emerson MR, Cross R, Nelson SR, Samson FE. Soman-induced seizures: limbic activity, oxidative stress and neuroprotective proteins. *J Appl Toxicol*. 2001:21(suppl 1):S87–S94.

161. Pagni CA, Zenga F. Posttraumatic epilepsy with special emphasis on prophylaxis and prevention. *Acta Neurochir Suppl*. 2005;93:27–34.

162. Yokoi I, Toma J, Liu J, Kabuto H, Mori A. Adenosines scavenged hydroxyl radicals and prevented posttraumatic epilepsy. *Free Radic Biol Med*. 1995;19:473–479.

163. Davies KJ. Oxidative stress: the paradox of aerobic life. *Biochem Soc Symp*. 1995;61:1–31.

164. Zivin M, Milatovic D, Dettbarn WD. Nitrone spin trapping compound N-tert-butyl-alpha-phenylnitrone prevents seizures induced by anticholinesterases. *Brain Res*. 1999;850:63–72.

165. Peterson SL, Purvis RS, Griffith JW. Comparison of neuroprotective effects induced by alpha-phenyl-N-tert-butyl nitrone (PBN) and N-tert-butyl-alpha-(2 sulfophenyl) nitrone (S-PBN) in lithium-pilocarpine status epilepticus. *Neurotoxicology*. 2005;26:969–979.

166. Cao X, Phillis JW. Alpha-Phenyl-tert-butyl-nitrone reduces cortical infarct and edema in rats subjected to focal ischemia. *Brain Res*. 1994;644:267–272.

167. Meyerhoff JL. Division of Neurosciences, Walter Reed Army Institute of Research, Silver Spring, Md 20910-7500, USA. Personal communication with Newmark J, March 2006.

168. Lockhart B, Roger A, Bonhomme N, Goldstein S, Lestage P. In vivo neuroprotective effects of the novel imidazolyl nitrone free-radical scavenger (Z)-alpha-[2-thiazol-2-yl)imidazol-4-yl]-N-tert-butylnitrone (S34176). *Eur J Pharmacol*. 2005;511:127–136.

169. Bauman RA, Yourick DL, Wessner K, et al. A model for studying neuroprotection from seizure-induced brain injury resulting from exposure to organophosphate (OP) nerve agents. In: Proceedings of the NATO Task Group on Prophylaxis and Therapy Against Chemical Agents; May 23–27, 2005. Hradec Kralove, Czech Republic.

170. Kudin AP, Debska-Vielhaber G, Vielhaber S, Elger CE, Kunz WS. The mechanism of neuroprotection by topiramate in an animal model of epilepsy. *Epilepsia*. 2004;45:1478–1487.

171. Setkowicz Z, Ciarach M, Guzik R, Janeczko K. Different effects of neuroprotectants FK-506 and cyclosporin A on susceptibility to pilocarpine-induced seizures in rats with brain injured at different developmental stages. *Epilepsy Res*. 2004;61:63–72.

172. Santos JB, Schauwecker PE. Protection provided by cyclosporin A against excitotoxic neuronal death is genotype dependent. *Epilepsia*. 2003;44:995–1002

173. Okonkwo DO, Melon DE, Pellicane AJ, et al. Dose-response of cyclosporin A in attenuating traumatic axonal injury in rat. *Neuroreport*. 2003;14:463–466.

174. Friberg H, Wieloch T. Mitochondrial permeability transition in acute neurodegeneration. *Biochimie*. 2002;84:241–250.

175. De Keyser J, Uyttenboogaart M, Koch MW, et al. Neuroprotection in acute ischemic stroke. *Acta Neurol Belg*. 2005;105:144–148.

176. Li J, Luan X, Lai Q, et al. Long-term neuroprotection induced by regional brain cooling with saline infusion into ischemic territory in rats: a behavioral analysis. *Neurol Res*. 2004;26:677–683.

177. Filbert M, Levine E, Ballough G. Neuroprotection for nerve agent-induced brain damage by blocking delayed calcium overload: a review. *Journal of Medical Chemical Biological and Radiological Defense*. 2005;3:1–21. Available at http://jmedcbr.org/Issue_0301/Filbert/Filbert_1105.pdf.