MANAGEMENT OF CONVULSIONS IN NERVE AGENT ACUTE POISONING: A POLISH PERSPECTIVE

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ABSTRACT

Symptoms of acute poisoning with nerve agents such as organophosphorus (OP) compounds include general convulsions that could lead to brain damage if uncontrolled. Seizures are believed to be due to hyperactivity of the cholinergic system as a result of inhibition of acetylcholinesterase (AChE). Evidence suggests that the seizure process could also be the result of activation of the N-methyl-d-aspartate (NMDA) system. Effects of various anticonvulsant drugs, such as classic antiepileptic drugs (barbiturates and phenytoin), agonists of the GABA inhibitory system (full and partial agonists of benzodiazepine [BDZ] receptors), antagonists of excitatory amino acid receptors (competitive and non-competitive NMDA receptor antagonists, aminomethylphosphonic acid [AMPA] receptor antagonists) are presented and discussed. Special attention is given to partial agonists of the BDZ recognition site, which produce high anticonvulsant activity with minor myorelaxant and sedative action. Of special interest is imidazenil, an imidazobenzodiazepine derivative, which may become the drug of choice in the management of convulsions in acute OP poisonings.

INTRODUCTION

Nerve agents or nerve gases are very toxic organophosphorus (OP) compounds. Tabun (ethyl N-dimethylphosphor-amidocyanidate; GA), Sarin (isopropyl methylphosphonofluoridate; GB), Soman (pinacolyl methylphosphonofluoridate; GD) and VX (O-ethyl-S-[2(diisopropylamino)ethyl] methylphosphonothioate) are nerve agents. OPs with lower toxicity to mammals are used as pesticides and tools in basic research. The nerve agents and many other OPs are very strong inhibitors of hydrolases, especially cholinesterases. Their main toxic effect is due to phosphorylation of the active site serine in acetylcholinesterase (AChE), which in mammals produces hyperactivity of the whole cholinergic nervous system due to the increase of endogenous acetylcholine (ACh) levels at neuronal synapses.

Symptoms of acute poisoning with OP include limbic seizures followed by general convulsions. This convulsive activity creates a problem for medical management and, if uncontrolled, can lead to brain damage. Shih et al. (1991) have investigated the mechanisms involved in the initiation and maintenance of convulsions caused by OP. They proposed a four-stage sequence of events: inhibitions of brain AChE leading to a rapid increase in brain ACh levels; interaction of excess ACh with cholinergic receptors (primarily muscarinic) to initiate cholinergic crisis; release of excitatory
neurotransmitters and loss of inhibitory transmission, triggered by a certain threshold of excess of ACh, and resulting in seizure activity in susceptible brain areas; and release of an excess amount of an endogenous substance (probably glutamate) such that it builds up to toxic concentrations and produces the consequent neuropathology.

The first two stages are relatively clear-cut, but the next two stages, involving excitatory and inhibitory neurotransmitters, are relatively uncertain. Effective management of OP-induced seizures is critical for both minimization of brain damage and full recovery from the central effects of exposure. Some studies (McDonough and Shih, 1993; Bodjarjan et al., 1993) have demonstrated that antimuscarinic drugs can block the onset of OP-induced seizures or terminate them when they are administered just after intoxication. These observations suggested that the cholinergic muscarinic mechanism predominated the initiation and early phase of OP-induced seizures. On the other hand, research has established that excitatory amino acids (EAA), such as glutamate, are released under some OP intoxications (e.g., Soman) and probably play a prominent role during seizures (Wade et al., 1987; Lallement et al., 1991).

Acute intoxications with OPs usually have been treated with repeated doses of cholinolytics (mostly atropine) along with repeated doses of an oxime to reactivate the AChE from the phosphorylated AChE (Gall, 1981). This combined regimen, at present, also includes a prophylactic pretreatment with pyridostigmine (Moore et al., 1995). Carbamates, such as pyridostigmine, carbamylate the active site serine in the same way as OPs, but the rate of spontaneous reactivation of the carbamylated AChE is faster than phosphorylated AChE. In that sense, pretreating with a carbamate can protect the AChE from inhibition by OP compounds. In many cases of poisoning with OP, this therapeutic regimen is insufficient and does not prevent or block seizure activity and convulsions (Lallement et al., 1997). Therefore, there is a need for more effective aniconvulsant therapy.

Classic anticonvulsant drugs, such as barbiturates or phenytoin, are not able to block the seizures or increase significantly the antilethal effectiveness of atropine in poisonings with OP (Wills, 1963). Diazepam can be given to mitigate the seizures in OP poisoning, but its sedative properties make it a less-than-optimal drug for use on the battlefield.

**REVIEW OF ANTICONVULSANT RESEARCH**

The first successful attempt to stop seizure activity and convulsions in OP poisonings was observed 30 years ago where the effects of diazepam were described (Lipp, 1972; Rump et al., 1972). Diazepam quickly abolished seizure bioelectrical activity in the rabbit’s brain induced by fluostigmine (diisopropyl fluorophosphate [DFP]) (Rump et al., 1973) or Soman (Johnson and Lowndess, 1974). When given together with atropine and obidoxime (an AChE reactivator), diazepam increased the effectiveness of that standard therapy in DFP intoxication two-fold (Rump et al., 1974).

In addition, diazepam has been shown to be efficacious not only against OP-induced convulsions, but also against subsequent neuropathological lesions (Martin et al., 1985). However, only administration of diazepam before the onset of convulsions completely prevented expression of pathology. If diazepam is administered at the start of, or at various times after the initiation of convulsions, the therapeutic benefit is
quickly lost (Clement and Broxup, 1993). These observations suggested that diazepam must be administered shortly after exposure to OP. Moreover, it was reported that seizure activity could redevelop within a few hours after diazepam administration when diazepam was given after the onset of seizures (McDonough and Shih, 1993). In our experiments, the subsequent administration of diazepam 90 min after the first dose was without effect on the LD$_{50}$ of DFP (Rump et al., 1976).

In addition, diazepam, as with other benzodiazepines (BDZ), has the potential to decrease performance when administered in anticonvulsant doses (Capacio et al., 1992). Diazepam also has anxiolytic, sedative, and muscle-relaxant properties. The last two properties are undesirable in nerve agent exposures.

An ideal anticonvulsant compound in OP poisonings should have minimal debilitatory side effects and sufficient water solubility for compatibility with current treatment regimens. Commercially available diazepam is formulated in a nonaqueous solvent. This is incompatible with the aqueous solutions of atropine and oximes and therefore presents several logistical complications. This technical inconvenience could now be overcome. Avizafone (a peptidoaminobenzophenone pro-drug of diazepam) is soluble in water (Upshall et al., 1990; Clement and Broxup, 1993) and could be used in a multiple aqueous drug mixture of atropine and oxime. Avizafone undergoes hydrolysis in the body by aminopeptidase to give lysine and diazepam (Maidment and Upshall, 1990). Effects of other BDZs were also studied. Some experiments suggested a higher efficacy of clonazepam (Lipp, 1974) compared to diazepam. In fact, the effectiveness of all anticonvulant BDZs are very similar (McDonough et al., 1999).

Despite all these counterindications, the anticonvulsant and antilethal effects and therapeutic value of diazepam in acute OP poisonings are generally accepted and diazepam, alongside with atropine and oxime, is often recommended as the drug of choice for the treatment of OP poisonings (Moore, 1995). As a result, in many countries, diazepam was made available in an automatic injector to counteract OP-induced convulsions. For example, the possibility for nerve agent use on the battlefield during Desert Storm resulted in the US Army providing soldiers with diazepam in auto-injectors.

However, sedation and dependence make diazepam a poor choice for prophylactic treatment, even though this drug is potentially life saving. If diazepam were used in combat conditions, especially in auto-injectors by non-professionals, a performance decrement and a decrease of fighting ability of the soldier, due to sedative and myorelaxant properties of this drug, could be a real consequence. This would occur in the absence of intoxication with OP (e.g., as a result of a false chemical alarm) or nonprescribed use of the injectors. Therefore, further research is needed to find a better antidote against OP-induced convulsions and associated debilitation.

**ANTAGONISTS OF NMDA RECEPTORS**

Recent evidence suggests that in late stages of intoxication with OP, non-cholinergic EAA receptors may become involved. A non-competitive antagonist of N-methyl-D-aspartate (NMDA) receptors, dizocilpine (5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate; MK-801) was used
effectively to block seizures induced by Soman in guinea pigs (McDonough and Shih, 1993; Braitman and Sparenborg, 1989; Sparenborg et al., 1992).

In studies of DFP intoxication, a competitive antagonist of NMDA receptors, CGP 39551 (carboxyethylester of DL-[E]-2-amino-4-methyl-5-phosphono-3-pentenoic acid), given as an adjunct to atropine and obidoxime, raised the effectiveness of obidoxime three-fold during 2 hours observation but was without effect after 24 hours observation (Galecka et al., 1995).

Lallement et al. (1994) reported that 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide disodium (NBQX), a selective inhibitor of non-NMDA receptors (coompetitive aminomethylphosphonic acid [AMPA] receptor agonist), can prevent or greatly reduce the epileptic activity resulting from Soman intoxication.

Interestingly, the phencyclidine derivative N-(1-[2-thienyl] cyclohexyl) piperidine (TCP), a NMDA receptor antagonist, was shown to neither prevent nor delay the onset of OP-induced seizures in Soman poisoning but did terminate or reduce these seizures (Carpentier et al., 1994). These observations led to the suggested use of TCP in the treatment of OP poisonings (i.e., co-administration of atropine, NBQX, and TCP) (Lallement et al., 1994a).

The effectiveness of dizocilpine and NBQX in preventing lethality in Soman poisoning was studied in our laboratory. The antilethal effects of dizocilpine were significantly weaker than those of diazepam; treatment with NBQX was more lethal than treatment without an anticonvulsant, using only atropine and the oxime HI-6 (1-(((4-amino-carbonyl)pyridino)methoxy)methyl)-2-(hydroxyimino)methyl)-pyridinium dichloride monohydrate), C_{14}H_{16}N_{4}O_{3.2}Cl) in Soman poisonings of mice (table 1) (Kowalczyk et al., 1997).
Table 1. Increase in LD$_{50}$ of Soman with various anticonvulsants in the mouse for 24h observation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LD$_{50}$ (µg/kg)$^\dagger$</th>
<th>TI$^\ddagger$</th>
<th>TE$^\S$</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soman</td>
<td>137 (126–150)</td>
<td>1</td>
<td>NA$^|$</td>
<td>Rump et al. (2001)</td>
</tr>
<tr>
<td>+ atropine + HI-6</td>
<td>856 (609–1204)</td>
<td>6.2</td>
<td>1</td>
<td>Rump et al. (2001)</td>
</tr>
<tr>
<td>+ atropine + HI-6 + diazepam (5 mg/kg)</td>
<td>1287 (808–1832)</td>
<td>9.4</td>
<td>1.5</td>
<td>Rump et al. (2001)</td>
</tr>
<tr>
<td>+ atropine + HI-6 + CGS 9896 (20 mg/kg)</td>
<td>1848 (1290–2114)</td>
<td>13.5</td>
<td>2.1</td>
<td>Kowalczyk et al. (1997)</td>
</tr>
<tr>
<td>+ atropine + HI-6 + imidazenil (2 mg/kg)</td>
<td>2140 (1640–2790)</td>
<td>15.6</td>
<td>2.5</td>
<td>Rump et al. (2001)</td>
</tr>
<tr>
<td>+ atropine + HI-6 + MK-801 (1 mg/kg)</td>
<td>1242 (902–1709)</td>
<td>9.1</td>
<td>1.4</td>
<td>Kowalczyk et al. (1997)</td>
</tr>
<tr>
<td>+ atropine + HI-6 + CGP 39551 (10 mg/kg)</td>
<td>1090 (674–1207)</td>
<td>7.9</td>
<td>1.3</td>
<td>Kowalczyk et al. (1997)</td>
</tr>
<tr>
<td>+ atropine + HI-6 + NBQX (30 mg/kg)</td>
<td>802 (696–923)</td>
<td>5.8</td>
<td>0.9</td>
<td>Kowalczyk et al. (1997)</td>
</tr>
</tbody>
</table>

$^\dagger$ Anticonvulsants were given intraperitoneally (i.p.) together with atropine (10 mg/kg) and HI-6 (70 mg/kg) immediately after the intoxication. Soman was given subcutaneously (s.c.)

$^\ddagger$ LD$_{50}$ = Lethal dose; dose that causes death in 50% of the animals tested

$^\S$ TI = Therapeutic index: LD$_{50}$ treated : LD$_{50}$ untreated animals

$^\|$ TE = Therapeutic efficacy: LD$_{50}$ treated : LD$_{50}$ animals treated only with atropine and HI-6

$^\|$ NA = Not applicable

OTHER ANTICONVULSANTS

McLean et al. (1992) tested the effects of memantine, an aminoadamantane derivative used in therapy of Parkinson’s disease and reported to be protective against maximal electroshock (MES) seizures in rats (Meldrum et al., 1986b). McLean et al. found that pretreatment and/or therapeutic administration of memantine with atropine reduced seizure intensity in rats intoxicated with Soman. However, further experiments by Koplovitz et al. (1997) did not confirm that initial positive finding.

Scopolamine also has been found to be efficacious against Soman-induced convulsions (Shih et al., 1991; Capacio and Shih, 1991). Anderson et al. (1994) suggested that scopolamine (at least in guinea pigs) could replace atropine or diazepam or both as therapy against Soman-induced incapacitation.

In another paper, Anderson et al. (1994) studied the efficacy of other anticholinergic drugs against Soman-induced convulsions. They found biperiden, a synthetic tertiary amine with strong atropine-like blocking effects in the CNS, also used for the treatment of Parkinsonian syndrome, to be of special interest. Biperiden has been reported to have anti-NMDA activity (Olney et al., 1987). In guinea pigs, biperiden and scopolamine were superior to diazepam in their effectiveness against Soman-induced convulsions and lethality (Anderson et al., 1994).
PREVENTING SEIZURES BY PRETREATMENT

Research has also been done on prophylactic measures against OP-induced seizures (i.e., on drugs that may be administered before OP intoxication). Reversible AChE inhibitors, mainly carbamates, have been used as a pretreatment for OP poisonings. These include pyridostigmine and physostigmine. Pyridostigmine, a quaternary ammonium carbamate derivative, does not cross the blood-brain barrier and therefore cannot protect against seizures and subsequent neuropathology (Grunwald et al., 1994). Physostigmine does cross the blood-brain barrier but has a very short half-life (Aquilonius and Hartvig, 1986) and high degree of individual variation in its bioavailability (Whelpton and Hurst, 1985). These characteristics make physostigmine unacceptable for pretreatment in OP intoxications.

Preliminary results of our experiments on the protective effects against OP-induced seizures with another reversible AChE inhibitor, donepezil, used in the treatment of Alzheimer’s disease (Thomas et al., 2001), suggested that this drug could be of practical value in the prophylaxis of OP poisonings. Donepezil, given prophylactically, decreased the intensity of bioelectrical seizure activity subsequently induced by Soman in the rat brain. Donepezil, given together with pyridostigmine, significantly reduced the lethal effects in mice poisoned with DFP or Soman (Rump et al., unpublished data).

Huperzine, an alkaloid isolated from the Chinese club moss Huperzia serrata, has been established as a slow, reversible inhibitor of AChE (Ashani et al., 1992). It was reported that huperzine gives some protection against OP-induced seizures and subsequent neuropathology (Lallement et al., 1997).

PARTIAL AGONISTS OF BDZ RECOGNITION SITES

Despite the progress in assessing the protective functions of these anticonvulsant drugs, it is not obvious which of these drugs are best to include in the treatment for OP-induced seizures. For that reason, we continued our research on possible anticonvulsants. We were especially interested in partial agonists of BDZ recognition sites. These drugs have anxiolytic effects, high anticonvulsant activity, and minor myorelaxant and sedative actions (Bernard et al., 1985; Meldrum and Chapman, 1986a).

A pyrazoloquinoline derivative, 2-p-chlorophenyl-pyrazolo 4,3-c-quinolin-3[5H]-one (CGS 9896), was studied as an anticonvulsant drug in OP poisonings. CGS 9896, given together with standard therapy consisting of atropine and obidoxime, was twice as effective as diazepam in mice poisoned with DFP during 24 hours observation (Rump et al., 1990). Similar effects for CGS 9896 were observed in Soman poisonings, where standard therapy consisted of atropine and HI-6 (table 1) (Kowalczyk et al., 1997). These observations, as well as lack of sedative and myorelaxant activity, suggested that CGS 9896 could be of value as an adjunct to cholinolytic drugs and AChE reactivators in the treatment of OP poisonings. Unfortunately, CGS 9896 has not been approved as a drug in any country.

Another partial agonist of the BDZ receptor, imidazenil (6-(2-bromophenyl)-8-fluoro-4H-imidazo[1,5-a]benzodiazepine-3-carboxamide), may be of interest. Imidazenil is
now extensively studied in many laboratories and clinics as a potential new-
generation anti-epileptic drug (Costa and Guidotti, 1996). Recently we reported the
effects of imidazenil in acute poisonings with DFP (Rump et al., 2000). This drug
significantly decreased convulsion intensity and seizure patterns in bioelectrical
activity of the brain and increased antilethal effectiveness of the standard therapy.
These effects were comparable to those of diazepam but had fewer side effects. Motor
coordination effects were noted in imidazenil at doses 5–10 times higher than
therapeutic doses, but these effects were noted in diazepam at therapeutic doses
(figure 1).

![Figure 1. Effects of imidazenil and diazepam in rota-rod test in the mouse](image)

Notes:
– Results were expressed as ability of performance (time to remain on the rod) according to Kuribara et
al. (1977).
– Proposed scores were defined as follows: 0: 0–4 s; 1: 5–9 s; 2: 10–14 s; 3: 15–19 s; 4: 20–24 s; 5: 25–
29 s; 6: > 30 s. (Rump et al., 2000).
– There was no DFP exposure.

Figure 1. Effects of imidazenil and diazepam in rota-rod test in the mouse

More recently we confirmed the efficacy of imidazenil in Soman intoxication (Rump
et al., 2001). The effectiveness of imidazenil, given together with atropine and HI-6,
was even higher than that of diazepam (table 1) for preventing death in mice. The
effects of imidazenil in the management of Soman-induced convulsions (figure 2) and
seizure bioelectrical activity in the brain (figure 3) were also very close to the effects
of diazepam.
Notes:
– To increase the survival rate, all animals received HI-6 (75 mg/kg i.p.) 15 min before the experiment.
– Intensity of convulsions was determined on Convulsometer (Columbus Instruments, USA) and expressed in g/sec (Rump et al., 2001).
– Imidazentil was given at 2 mg/kg i.p.
– Diazepam was given at 5 mg/kg i.p.
– Soman was given at 200 µg/kg s.c.

Figure 2. Effects of imidazentil or diazepam on convulsions induced by Soman in the mouse
Notes:
- To increase the survival rate, all animals received HI-6 (80 mg/kg i.p.) and methylatropine (10 mg/kg i.p.) 15 min before the experiment.
- Four stages of intensity of seizure were determined according to Lallement et al. (1994): stage 1 – absence of spikes and sharp waves; stage 2 – discrete spikes and sharp waves on normal background; stage 3 – high voltage spikes and sharp waves on a suppressed background; stage 4 – continuous or bursting high voltage spiking (Rump et al., 2001).
- Imidazenil was given intraperitoneally at 5 mg/kg
- Diazepam was given intraperitoneally at 5 mg/kg
- Soman was given subcutaneously at 180 µg/kg

Figure 3. Effects of imidazenil or diazepam on seizure bioelectrical activity of the rat brain induced by Soman

However, imidazenil is still not approved and registered as a drug. But if the clinical studies conducted at present, especially those concerning the chronic toxicity, confirm the initial positive finding and if imidazenil becomes registered, it could become a drug of choice for the management of convulsions in OP poisonings.

CONCLUSIONS

Controlling the seizures induced by OP poisoning is an essential part of medical treatment. It is critical to give the anticonvulsant drug as soon as possible after OP exposure. For this reason, it is important to have the drug available in the field for soldiers to administer to themselves, along with atropine and a cholinesterase regenerator, such as HI-6. Diazepam is currently either fielded or suggested for use in many countries. Administration of this drug in the absence of OP exposure can cause
the degradation of the soldier’s motor coordination and other effects; in addition, there have been documented risks of abuse of the diazepam. Consequently, based on our experiments using imidazenil, we are suggesting that imidazenil is of similar effectiveness to diazepam but has fewer unwanted side effects. This may permit the use of this drug in combat conditions without decreasing the fighting ability of the soldier.

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References


