USE AND RISKS OF ANTICONVULSANT THERAPY IN NERVE AGENTS POISONINGS IN COMBAT CONDITIONS

S. RUMP, M. KOWALCZYK, O. ANTKOWIAK, E. GIDYNSKA, E. GALECKA
Dept. Pharmacology and Toxicology, Military Institute of Hygiene and Epidemiology, Warsaw, Poland

Centrally mediated seizures are one of the toxic signs that occur following poisoning with a very toxic organophosphate (OP) soman (pinacolyl methyl phosphonofluoridate). They are belived to be primarily due to hyperactivity of the cholinergic system as a result of decreased acetylcholinesterase (AChE; EC 3.1.1.7) activity and increased endogenous acetylcholine (ACh) level at central neuronal synapses.

This convulsive activity creates a problem for medical management and, if uncontrolled, can lead to brain damage. A combined regimen of prophylaxis and therapy, consisting of pretreatment with pyridostygmine and treatment with atropine and oxime (mostly HI-6), is now generally agreed to be the most effective medical countermeasure for dealing with these poisonings (Dunn and Sidell, 1989; Moore *et al.,* 1995).

However, this combined treatment regimen does not appear to block completely seizure activity and concomitant motor convulsions (Leadbeater *et al., 1*985). In many experimental animals these seizures rapidly progress to status epilepticus and contribute to the profound brain damage (Lemercier *et al.,* 1983; McLeod *et al.,* 1984; Petras, 1994).

Therefore effective management of soman-induced seizures is critical for both minimalization of brain damage and full recovery from the central effects of exposure. Undoubtedly, several anticholinergic drugs (like scopolamine) can block in some cases the onset of OP-induced seizures (Capacio and Shih, 1991; Anderson *et al.,* 1994). However, effectiveness of these drugs is limited and in many cases they do not prevent the onset of convulsions (McDonough and Shih, 1993).

Benzodiazepines, especially diazepam, are very effective in the treatment of OP-induced convulsions when given as adjuncts to atropine and oxime (Lipp, 1972, 1973; Rump *et al.,* 1972, 1973; Johnson and Lowndes, 1974; and others) Although neuropathology was significantly reduced compared with animals that did not receive diazepam, the incidence and degree of protection afforded were never complete. Administration of diazepam before the start of convulsions prevented expression of the pathology, whereas, if diazepam was administered either at the start of or at various times after the initiation of convulsions the therapeutic benefit was quickly lost (Clement and Broxup, 1993).

These observations suggested that the use of diazepam must be initiated shortly after the exposure to soman in order for any therapeutic benefit to be realised. Otherwise administration of diazepam would only control the seizures without a providing therapeutic benefit against the neuropathology.

Diazepam is now commonly used for the management of convulsions in OP intoxications. In many countries diazepam is provided to military forces in a special autoinjectors to deliver the drug i.m. by ordinary soldier as quickly as possible after the contact with OP (Moore *et al.,* 1995).

Unfortunately, diazepam, alongside anticonvulsant and anxiolytic activity has sedative and myorelaxant properties. Therefore when diazepam would be used in combat conditions in autoinjector by non-professional people without real intoxication with OP (e.g. as a result of a false chemical alarm) 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.

From the military point of view there is a need to search other anticonvulsant drugs which could control the OP-induced seizure activity and do not produce the unwanted side-effects and performance decrement.

Partial benzodiazepine receptor agonists are regarded as producing sedation and ataxia only when given in very high doses (Bernard *et al.,* 1985). Imidazenil, recently reported imidazobenzodiazepine derivative (Giusti *et al.,* 1993) and partial allosteric modulator of benzodiazepine receptors seems to be of special interest.

Recently we reported the effects of imidazenil in acute poisonings with DFP (Rump *et al.,* 2000). Now, we tried to determine whether imidazenil could be used effectively and safely as anticonvulsant also in acute poisonings with soman.

Material and Methods

Experiments were performed on Swiss strain male mice and Wistar strain male rats housed in a temperature controlled room (23 ± 1 °C) with free access to standard food and water. Experiments were carried out in accordance with the requirements of the Polish State Animal Protection Act (Scientific Procedures) and experimental design was approved by the local Ethical Committee of the Institute. Soman (pinacolyl methylphosphonofluoridate) of 98% purity was used. It was dissolved in propylene glycol to 10% concentration and kept at 4 °C. This stock solution was diluted to the desired concentration with redistilled water just before the experiment. Imidazenil (6-(2-bromophenyl)-8-fluoro-4H--imidazo[1,5-a]benzodiazepine-3-carboxamide) was dissolved in a solvent proposed for diazepam by Crankshow and Raper, 1971). Compound HI-6 ([[[(4-aminocarbonyl) -pyridino] methoxy]methyl]--2-[(hydroxyimino)methyl] pyridinium dichloride) and atropine sulphate were dissolved in redistilled water and diazepam (Valium - Hoffmann-LaRoche) was used in the original solution as supplied.

Anticonvulsant Efficacy

Effects on convulsions intensity were determined on mice using convulsometer (Columbus Instruments, USA). The control group received soman (200 μg/kg s.c.) and in order to increase the survival rate HI-6 (75 mg/kg i.p.). Experimental groups additionally received imidazenil (2 mg/kg i.p.) or diazepam (5 mg/kg i.p.) immediately after the intoxication. Intensity of convulsions was measured at 10, 30, 60 and 120 min after the treatment and expressed in g/s.

Effects on seizure bioelectrical activity of the brain was studied on rats with chronically implanted cortical stainless steel electrodes. Animals were divided into three groups (control and two experimental). Bioelectrical activity was registered every 5 min for 30 min using Grass Model 78 Polygraph in control group receiving soman (180μg/kg i.p.) and to increase the survival rate HI-6 (80 mg/kg i.p.) and methylatropine (10 mg/kg i.p.). Experimental groups were treated additionally with imidazenil (5 mg/kg i.p.) or diazepam (5 mg/kg i.p.). Four stages of intensity of seizure activity was determined according to Lallement et al. (1994):

- stage 1 – absence of spikes or sharp waves;
- stage 2 – discrete spikes and sharp waves on a normal background;
- stage 3 – high voltage spikes and sharp waves on a suppressed background;
- stage 4 – continuous or bursting high voltage spiking.

Antilethal Efficacy

Antilethal effectiveness was examined on mice as the influence of imidazenil (2 mg/kg i.p.) or diazepam (5 mg/kg i.p.) on the efficacy of standard therapy consisted of atropine (10 mg/kg i.p.) and HI-6 (75 mg/kg i.p.) by means of determination of LD_{50} value of soman given s.c. for 2 and 24 h observation using the method decribed by Thompson (1947).

Statistical Analysis

Data analysis was performed using Student's *t*-test for individual comparisons with the exception of antilethal efficacy when Litchfield-Wilcoxon method with the use of program set up by Tallarida and Murray (1987) was employed. A *p*-value of 0.05 ort less was required for significance.

Results

Effects on convulsions intensity of imidazenil given in a dose of 2 mg/kg i.m. were comparable with the effects of diazepam given in a dose of 5 mg/kg i.m. (Fig. 1). Both drugs diminished the intensity of soman-induced convulsions in the mouse immediately after application and their effects could be observed within 2 h.

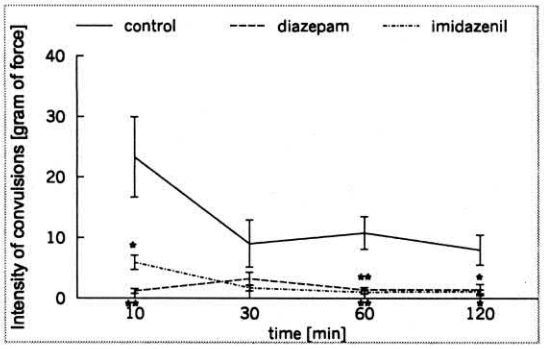

Fig. 1: Effects of imidazenil (2 mg/kg i.p.) and diazepam (5 mg/kg i.p.) on convulsions induced by soman (μg/kg s.c.) in the mouse.

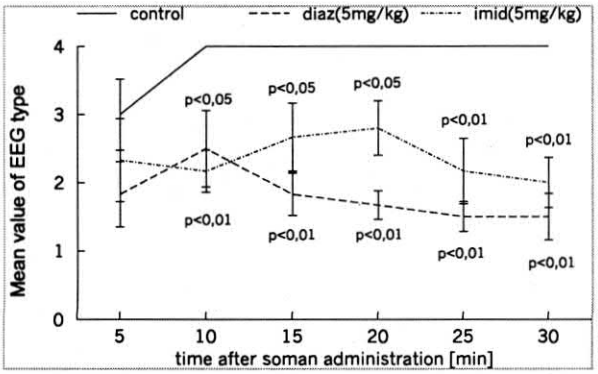

Fig. 2: Effects of imidazenil (5 mg/kg i.m.) and diazepam (5 mg/kg i.m.) on seizure bioelectrical activity of the rat brain induced by soman (180 μg/kg i.p.).

Administration of soman (180 μg/kg i.p.) to the rat resulted within 10 min in a spike activity in the cortex (mean value of intensity 4) which lasted to the end of experiment, i.e. till 30 min. Administration of imidazenil (5 mg/kg) decreased the intensity of seizure activity significantly (Fig. 2). Effects of diazepam (5 mg/kg) were similar though a little bit higher.

Table 1

Effects of Tested Drugs on LD_{50} of Soman (μg/kg) in the Mouse During 2h Observation

	LD_{50}	TI	TE
SMN	137 (126–150)	1	-
+ A + HI-6	1216 (1165–1268)	8.9	1
+ A + IH-6 + Imidazenil	4415 (3386–5750)	32.2	3.6
+ A + IH-6 + Diazepam	3180 (2461–4103)	23.2	2.6

Table 2

Effects of Tested Drugs on LD_{50} of Soman (μg/kg) in the Mouse During 24h Observation

	LD_{50}	TI	TE
SMN	137 (126–150)	1	-
+ A + HI-6	856 (609–1204)	6.2	1
+ A + IH-6 + Imidazenil	2140 (1640–2790)	15.6	2.5
+ A + IH-6 + Diazepam	1287 (808–1832)	9.4	1.5

Antilethal efficacy in the mouse after 2 h observation is shown in the Table 1 and after 24 h observation in the Table 2. Imidazenil (2 mg/kg i.p.) and diazepam (5 mg/kg i.p.) when given as adjuncts to the standard therapy consisted of atropine and HI-6 increased the effectiveness of the therapy for 2h observation 3.6 or 2.6 times, respectively, and for 24h observation 2.5 and 1.5 times, respectively.

Discussion

Diazepam is now commonly used for the management of convulsions in OP intoxications and in many countries is included into special antichemical kits issued to military personnel (Moore *et al.*, 2000). However, sedation and myorelaxation made diazepam a not so optimal choice for treatment of convulsions in these situations. This is of special importance when diazepam is administered to a person who was not intoxicated with OP. If we decide to give diazepam into the hands of ordinary soldier we should be aware that the incapacitation of the soldier after improper use of the drug is a real consequence. Our present results showed that effectiveness of imidazenil in the management of soman-induced convulsions is very close to the effects of diazepam. Antilethal effects of imidazenil is even higher than that of diazepam when these drugs are given as adjuncts to the standard therapy in poisonings with soman. Effects of imidazenil and diazepam on motor co-ordination of the mouse in rota-rod treadmill were reported elsewhere (Rump *et al.*, 2000). It was stated that diazepam in therapeutic doses (5 mg/kg i.p. in the mouse) elicited strong effects and produced ataxia when imidazenil decreased only slightly the performance ability in doses higher than 10 mg/kg. To achieve the same level of effects of diazepam at 5 mg/kg a dose of imidazenil at 25 mg/kg was needed, i.e. a dose > 10 times higher than therapeutic dose (2 mg/kg in the mouse).

However, imidazenil in any country is not approved and registered as a drug. Imidazenil, according to Costa and Guidotti (1996) is considered as a potential antiepileptic drug of new generation and is now under the extensive clinical trials.If further studies, especially those concerning the chronic toxicity, confirm the positive initial opinion and imidazenil would be registered it could become a drug of choice for the management of convulsions in OP intoxications. This is not only because of similar effectiveness to diazepam but especially because of lack of unwanted central effects of imidazenil which allows to use this drug in combat conditions unafraid to decrease the fighting ability of the soldier.

References

1. ANDERSON, DR. – GENNINGS, C. – CARTER, WH., et al.: Efficacy comparison of scopolamine and diazepam against soman-induced debilitation in guinea-pig. *Fund. Appl. Toxicol.*, 1994, 22, p. 588–593.
2. BERNARD, PS. – BENNETT, DA. – PASTOR, B., et al.: CGS 9896 agonist-antagonist benzodiazepine receptor activity revealed by anxiolytic, anticonvulsant and muscle relaxation assesment in rodents. *J. Pharmacol. Exp. Ther.*, 1985, 235, p. 98–105.
3. CAPACIO, BR. – SHIH, T-M.: Anticonvulsant actions of anticholinergic drugs in somman poisoning. *Epilepsia*, 1991, 32, p. 604–615.
4. CLEMENT, JG. – BROXUP, B.: Efficacy of diazepam and avizafone against soman-induced neuropathology in brain of rats. *Neurotoxicol.*, 1993, 14, p. 485–504.
5. COSTA, E. – GUIDOTTI, A.: Benzodiazepines on trial: a research strategy for their rehabilitation. *TIPS*, 1996, 17, p. 192–200.
6. CRANKSHOW, DP. – RAPER, C.: The effects of solvents on the potency of chlordiazepoxide, diazepam, medazepam and nitrazepam. *J. Pharmacol. Pharm.*, 1971, 23, p. 313–321.
7. DUNN, MA. – SIDELL, FR.: (1989) Progress in medical defence against nerve agents. *JAMA*, 262, p. 649–652.
8. GIUSTI, P. – DUCIC, I. – PUIA, G., et al.: Imidazenil: a new partial positive allosteric modulator of γ-amino-butyric acid (GABA) action at $GABA_A$ receptors. *J. Pharmacol. Exp. Ther.*, 1993, 266, p. 1018–1028.
9. JOHNSON, DD. – LOWNDES, HE.: Reduction by diazepam of repetitive electrical activity and toxicity resulting from soman. *Eur. J. Pharmacol.*, 1974, 28, p. 245–250.
10. LALLEMENT, G., et al.: Antiepileptic effects of NBQX against soman-induced seizures. *NeuroReport*, 1994, 54, p. 25–428.
11. LEADBEATER, L. – INNS, RH. – RYLANDS, JM.: Treatment of poisoning by soman. *Fund. Appl. Toxicol.*, 1985, 5, p. S225–231.
12. LEMERCIER, G., et al.: Histological and histochemical changes in the central nervous system of the rat poisoned by an irreversible anticholinesterase organophosphorous compound. *Acta Neuropathol., (Berlin)*, 1983, 61, p. 123–129.
13. LIPP, JA.: Effects of diazepam upon soman-induced seizure activity and convulsions. *Electroenceph. Clin. Neurophysiol.*, 1972, 32, p. 557–569.
14. LIPP, JA.: Effects of benzodiazepine derivatives on soman-induced seizure activity and convulsions in the monkey. *Arch. int. Pharmacodyn.*, 1973, 202, p. 244–251.
15. McDONOUGH, JH. – SHIH, T-M.: Pharmacological modulation of soman-induced seizures. *Neurosci. Behav. Rev.*, 1993, 17, p. 203–215.
16. McLEOD, CG. – SINGER, AW. – HARRINGTON, DG.: Acute neuropathology in soman poisoned rats. *Neurotoxicology*, 1984, 5, p. 53–58.
17. MOORE, DH., et al.: Review of nerve agents inhibitors and reactivators of acetylcholinesterase. In QUINN, DM., et al. (Eds), *Enzymes of Cholinesterase Family* Plenum Press, New York, 1995, p. 297–304.
18. PETRAS, JM.: Neurology and neuropathology of soman-induced brain injuries. *J. Exp. Anal. Behav.*, 1994, 61, p. 319–329.
19. RUMP, S. – GRUDZINSKA, E. – EDELWEJN, Z.: Effects of diazepam on abnormsalities of bioelectrical activity of the rabbit's brain due to fluostigmine. *Activ. Nerv. Sup. (Prague)*, 1972, 14, p. 176–177.
20. RUMP, S. – GRUDZINSKA, E. – EDELWEJN, Z.: Effects of diazepam on epileptiform patterns of bioelectrical activity of the rabbit's brain induced by fluostigmine. *Neuropharmacology*, 1973, 12, p. 813–817.
21. RUMP, S. – GIDYNSKA, T. – GALECKA, E., et al.: Effects of imidazenil, a new benzodiazepine receptor partial agonist, in the treatment of convulsions in organophosphate intoxications. *Neurotoxicity Res.*, 2000, 2, p. 17–22.
22. TALLARIDA, RJ. – MURRAY, RB.: *Manual of Pharmacological Calculations with Computer Programs.* Springer Verlag. New York - Berlin - Heidelberg - London - Paris - Tokyo, 1987, p. 153–158.
23. THOMPSON, WR.: Use of moving averages and interpolation to estimate median effective dose. *Bac. Rev.*, 1947, 11, p. 115–145.

Correspondence: S. Rump
Department of Pharmacology and Toxicology
Military Institute of Hygiene and Epidemiology
Warsaw
Poland

Received: 11. 10. 2001