

## **46. IMIDAZENIL, A PROPOSED DRUG FOR THE TREATMENT OF CONVULSIONS IN ACUTE POISONINGS WITH ORGANOPHOSPHATES**

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### **SUMMARY**

Anticonvulsant and antilethal effects of imidazenil, a new imidazobenzodiazepine derivative, in soman acute poisonings were studied and compared to the effects of diazepam on mice and rats. It was stated that imidazenil in comparable manner to diazepam decreased intensity of convulsions, inhibited seizure bioelectrical activity and increased antilethal effectiveness of atropine and HI-6 in animals intoxicated with soman. These effects of imidazenil were noted in doses 5-10 times lower than those which influence the motor co-ordination when therapeutic doses of diazepam produce serious disturbances in motor co-ordination.

### **INTRODUCTION**

Symptoms of acute poisonings with organophosphates (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. A combined regimen of prophylaxis and therapy, consisting of pretreatment with pyridostigmine and treatment with atropine and oxime, is now generally accepted as the most effective.

However, this combined treatment regimen does not appear to block OP-mediated seizure activity and concomitant motor convulsions. In experimental animals these seizures rapidly progress to status epilepticus and contribute to the profound brain damage (1).

Classic anticonvulsant drugs are not capable to increase significantly the antilethal effectiveness of atropine in OP intoxications. Therefore the first successful attempt to break convulsions in OP intoxications was introduction of benzodiazepines (BDZ) into the therapy (2,3,4).

BDZ, especially diazepam, could block or terminate OP-induced convulsions, reduce neuropathology, and enhance survival, especially when given in conjunction with carbamate pretreatment and atropine and oxime therapy.

Although neuropathology was significantly reduced compared with animals that did not receive diazepam the incidence and degree of protection afforded were never complete (5). 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.

From the practical point of view it means that administration of diazepam must be initiated shortly after OP exposure, before the onset of convulsions, in order for any therapeutic benefit to be realized.

Diazepam alongside anticonvulsant activity has anxiolytic, sedative and myorelaxant properties which make it has potential to produce performance decrement when administered in anticonvulsant doses (6). This is of special importance when diazepam was administered to a person who was not intoxicated (e.g. as a result of a false chemical alarm). Therefore research should be continuing to find a better antidote against OP-induced convulsions and associated debilitation.

Partial BDZ receptor agonists are regarded as producing these side effects only in very high doses. Recently reported partial allosteric modulator of BDZ receptors, imidazenil (7) seems to be of special interest regarding the management of OP-induced convulsions.

Present study was performed in order to determine anticonvulsant and antilethal effects of imidazenil in acute poisonings with soman, a very toxic OP compound, and compare them with the effects of diazepam.

### **MATERIAL AND METHODS**

Experiments were performed on Swiss strain mice (weighing 20-25 g) and Wistar strain rats (weighing 200-250 g) obtained from the Institute's own Animal Farm, with free access to standard food and tap water and in 12 h light/dark cycle. 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 methyl phosphonofluoridate) was used as a model OP compound. It was dissolved in propylene glycol to 100 mg/ml and kept at 4°C. This stock solution was diluted to the desired concentration with redistilled water just before the experiment. Imidazenil and diazepam in crystal form were dissolved in a solvent proposed by Crankshaw and Raper (8). Atropine sulphate and HI-6 in crystal form were dissolved in redistilled water.

Anticonvulsant efficacy was determined as:

(i) effects on intensity of convulsions induced in the mouse by s.c. administration of soman (200 µg/kg s.c.) and measured on Convulsometer (Columbus Instruments, USA). All animals in order to increase the survival rate received HI-6 (75 mg/kg i.p.). Experimental groups received imidazenil (2 mg/kg i.p.) or diazepam (5 mg/kg i.p.) immediately after the intoxication. Intensity of subsequent convulsions was measured at 10, 30, 60 and 120 min after the treatment and expressed in g/sec., and

(ii) effects on seizure bioelectrical activity of the brain due to administration of soman (180 µg/kg s.c.). Experiments were performed on rats with chronically implanted cortical stainless steel electrodes. Bioelectrical activity was registered every 5 min for 30 min using Grass Model 78 Polygraph. Four stages of intensity of seizure activity was determined according to Lowenstein et al (9): 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. Experimental groups received imidazenil (5 mg/kg i.p.) or diazepam (5 mg/kg i.p.). All animals received HI-6 (80 mg/kg i.p.) and methylatropine bromide (10 mg/kg i.p.) in order to increase the survival rate.

Antilethal efficacy was examined on mice as the influence of imidazenil (2 mg/kg i.p.) or diazepam (5 mg/kg i.p.) on the value of LD<sub>50</sub> of soman given s.c. for 24 h observation and compared to the effectiveness of the standard therapy consisted of atropine (10 mg/kg i.p.) and HI-6 (75 mg/kg i.p.), using Thompson's method (10).

Statistical analysis was performed using Student's t-test for individual comparisons to evaluate the significance of means with the exception of antilethal efficacy when Litchfield-Wilcoxon method with the use of program set up by Tallarida and Murray (11) was employed. A p-value of 0.05 or less was required for significance.

## RESULTS AND DISCUSSION

Doses of imidazenil or diazepam used in experiments were established previously (12).

Effects on convulsions intensity were depicted on the Figure.1. Effects of imidazenil were similar to those of diazepam. Both drugs diminished the intensity of convulsions due to soman (200 µg/kg) in the mouse immediately after application and their effect could be observed within 2 hrs.

Administration of soman in a dose of 180 µg/kg to the rats pretreated with HI-6 resulted within 10 min in a spike activity in the cortex (mean value of intensity stage 4), which lasted to the end of experiment, i.e. till 30 min. Administration of imidazenil or diazepam decreased the intensity of seizure activity (mean value for imidazenil 2.1, for diazepam 1.8) (Fig.2). Complete normalization of the record was not seen during the observation period.

Antilethal efficacy in the mouse was shown in the Table 1. Imidazenil in a dose of 2 mg/kg given as adjunct to standard therapy consisted of atropine (10 mg/kg) and HI-6 (75 mg/kg) resulted in an increase of the effectiveness of the therapy 3.6 times for 2hrs observation and 2.5 times for 24 hrs observation when effectiveness of diazepam (5 mg/kg) was 2.6 times and 1.5 times respectively.

Comparison of the effects of imidazenil and diazepam on performance of mice in Rota-rod treadmill was reported recently from our laboratory (12). Imidazenil was decreasing the performance ability in doses higher than 10 mg/kg when diazepam elicited strong effects on motor co-ordination in a dose of 5 mg/kg. To achieve the same level of effects as diazepam at 5 mg/kg a dose of imidazenil at 25 mg/kg was needed.

Diazepam is now commonly used for the management of convulsions in OP intoxications. In many countries diazepam is provided to military forces in special autoinjectors to deliver the drug i.m. by ordinary soldier as quickly as possible after the contact with OP (13).

However, when autoinjectors are used 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 diazepam could be a real consequence.

Our present results indicate that effectiveness of imidazenil in the management of soman-induced convulsions is very close to the effects of diazepam. Effects of imidazenil on soman-induced seizure bioelectrical activity of the brain are also comparable to those of diazepam. And antilethal effects are even higher than those of diazepam.

However, diazepam in therapeutic doses (from 2 mg/kg in the mouse) produces very strong disturbances in motor coordination. Such an effect was observed after the administration of imidazenil in a dose of 25 mg/kg, i.e. > 10 times higher than therapeutic dose (2 mg/kg in the mouse). This is a very great practical advantage of imidazenil.

However, imidazenil in any country is not approved and registered as a drug. Imidazenil, according to Costa and Guidotti (14) is considered as a potential antiepileptic drug of new generation. 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.

## ACKNOWLEDGEMENTS

This work was supported by a grant from the State Committee for Scientific Research (KBN) under the contract No

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**KEYWORDS**

convulsions, diazepam, imidazenil, organophosphates, Soman

**FIGURES AND TABLES**

Figure 1. Effects of imidazenil or diazepam on convulsions due to soman (200 µg/ kg sc) intoxication in the rat.

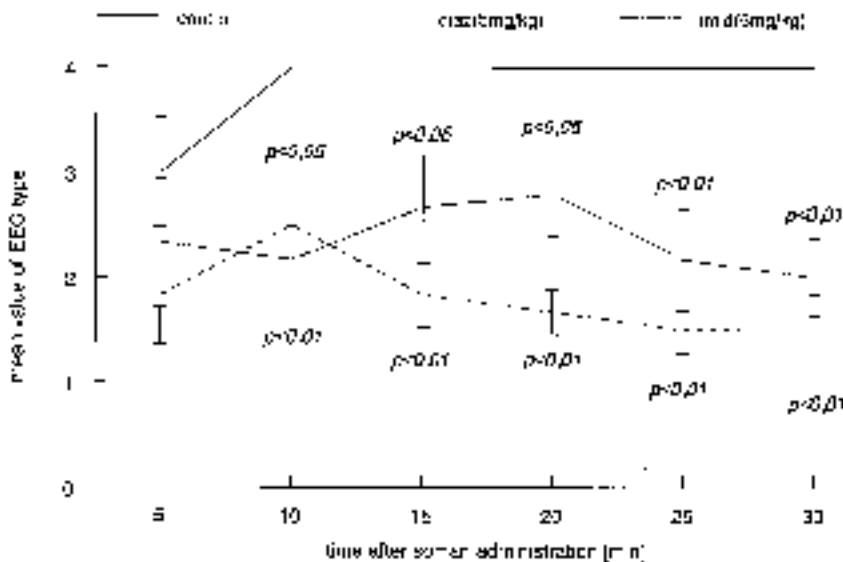
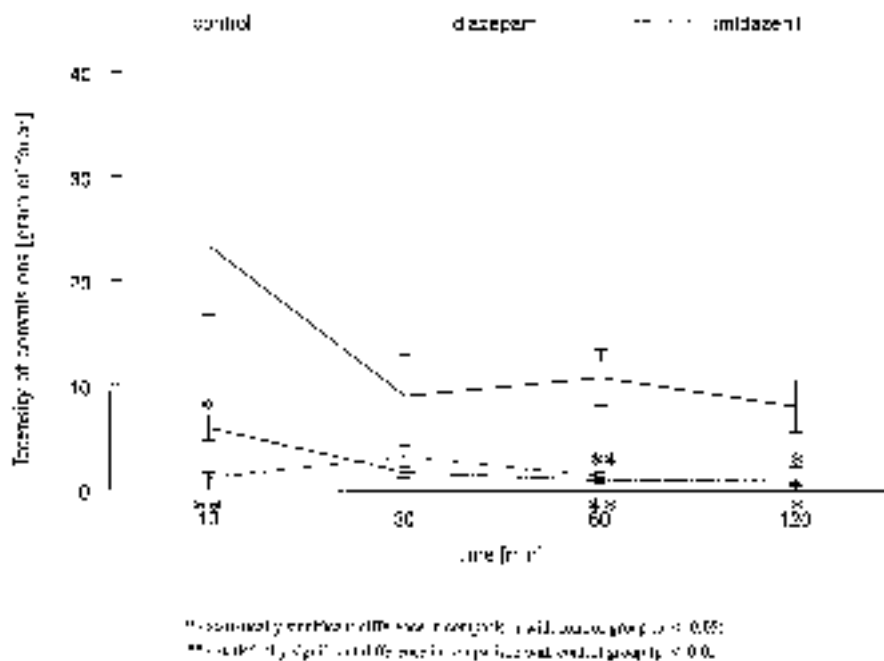


Figure 2. Effects of imidazenil or diazepam on the soman (180 µg/ kg sc) induced seizure bioelectrical activity of the rat brain.



**Table 1.** Antilethal effects of diazepam (5 mg/kg ip) or imidazenil (2 mg/kg ip) given together with atropine (10 mg/kg ip) and HI-6 (75 mg/kg ip) in soman (µg/ kg sc) intoxication in the mouse.

	<b>LD 50</b>	<b>TE *</b>
<b>soman</b>	137(126-150)	1
<b>soman + atropine + HI-6</b>	856 (609-1204)	6.2
<b>soman + atropine + HI-6 + diazepam</b>	1287 (808-1832)	9.4
<b>soman + atropine + HI-6 + imidazenil</b>	2140 (1640-290)	15.6

\* Therapeutic efficacy = LD 50 with treatment : LD 50 without treatment  
 In parentheses confidence limits

