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ANTICONVULSIVE AND PROTECTIVE EFFECTS OF DIAZEPAM AND MIDAZOLAM IN RATS POISONED BY HIGHLY TOXIC ORGANOPHOSPHORUS COMPOUNDS

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The aim of this study was to compare the anticonvulsive and protective effects of diazepam and midazolam in rats poisoned by chemical warfare agents. In rats treated with soman, sarin or VX, the anticonvulsive effects of midazolam and diazepam were of similar magnitude. Atropine and oxime HI-6 decreased the toxicity of soman, sarin and VX 1.65, 2.06 and 18.3 times, respectively. The introduction of diazepam and midazolam in the therapy of rats poisoned by VX and sarin led to further improvement of protective indices. Midazolam was even more effective than diazepam. A reliable protective effect was obtained with the lowest dose of both benzodiazepines used (0.5 mg/kg). The specific benzodiazepine antagonist flumazenil abolished, almost completely, the protective effect of both benzodiazepines. These data confirmed a significant role of the gabaergic system in poisoning with organophosphorus compounds, especially during the initial stage of intoxication.

Key terms: anticonvulsants, antidotes, atropine, benzodiazepines, chemical warfare agents, current therapy of intoxication, flumazenil, oxime HI-6, sarin, soman, sublethal doses, VX.

The current therapy of intoxication with the majority of organophosphate (OP) compounds is administration of atropine and an oxime. As OP compounds can provoke seizures that are not easily controlled by atropine, the introduction of anticonvulsants in therapy is necessary. It has been reported that the experimental treatment of fluostigmine poisoning could be improved by the use of diazepam (1), which is also recommended for human use in the treatment of nerve agent poisoning (2). According to some investigations the water-soluble benzodiazepine midazolam provides even better protection than diazepam in rats poisoned by paraoxon (3, 4).

The aim of this study was to compare the anticonvulsive and protective effects of midazolam (8-chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo-(1,5-a) (1, 4) benzodiaze-

pine maleate) and diazepam in rats poisoned with soman, sarin or VX and to establish their most efficacious doses in OP intoxication. In order to obtain more detailed information about the protective effectiveness of benzodiazepines, the benzodiazepine receptor antagonist flumazenil (ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H--imidazo(1,5-a) (1, 4) benzodiazepine-3-carboxylate was used.

MATERIALS AND METHODS

All experiments were carried out on male Wistar rats, weighing 180–200 g. The rats had free access to food and water before and after drug administration.

Anticonvulsive effect of benzodiazepines

Increasing doses of midazolam and diazepam were administered 10 min before a subcutaneous injection of OP compounds. To evaluate the anticonvulsive effects of benzodiazepines all the animals were monitored for the persistence of clonic-tonic movements within the period of 60 min after the poisoning. The number of rats per group having visible signs of seizure activity served as a parameter for the calculation of ED_{50} curves by probit analysis.

Estimation of LD_{50} values

The antidotes were administered by an intramuscular injection immediately before a subcutaneous injection of OP compounds. Six animals per dose and at least four different doses were used in constructing the LD_{50} curves. Twenty-four-hour LD_{50} values were calculated by probit analysis according to the method of *Litchfield and Wilcoxon* (5). The level of protection was expressed by means of the protective index (LD_{50} with treatment/LD_{50} without treatment). Throughout the experiments LD_{50} values of soman, sarin and VX were: 80, 150 and 16 μg/kg s.c. Soman, sarin and VX were synthesized in the Chemical Department of the Military Technical Institute. Oxime HI-6 (2-hydroxyiminomethylpyridinium-1-methyl-4'-carbamoyl-pyridinium-1'-methyl ether dichloride monohydrate) was supplied by Bosnalijek, Sarajevo. Solutions of all poisons and antidotes, except for diazepam and flumazenil, were prepared in 0.9% NaCl. Diazepam was prepared in a mixture of 40% propylene glycol, 10% ethyl alcohol and 50% distilled water. Flumazenil (10 mg/kg) was initially dissolved in distilled water and finally prepared for injection by adding a few drops of Tween 20. Fixed doses of atropine (10 mg/kg) and oxime HI-6 (10 mg/kg) were used in all experiments. Various doses of either diazepam (0.5, 1.0, 2.5, 5.0 mg/kg) or midazolam (0.5, 1.0, 2.5, 5.0 mg/kg) were given in combination with atropine and oxime. All injections were given separately in volume ml/kg.

Statistical procedure

All statistical comparisons (5) having a P value <0.05 were considered to be significant.

RESULTS

The anticonvulsive effects of diazepam and midazolam in rats poisoned with sublethal doses of soman, sarin and VX are shown in Table 1. Both benzodiazepines exhibited a similar degree of anticonvulsive activity irrespectively of the OP

Table 1.

Anticonvulsive effects of midazolam and diazepam in soman, sarin and VX treated rats

OP compounds μg/kg	Benzodiazepines	ED_{50} μmol/kg	Relative potency
Soman	Diazepam	6.1	1.0
70.0	Midazolam	8.0	0.75
Sarin	Diazepam	9.0	1.0
130.0	Midazolam	7.5	1.2
VX	Diazepam	15.3	1.0
14. 0	Midazolam	9.1	1.7

Benzodiazepines were administered 10 min. before s.c. injections of OP compounds.

Table 2.

Effects of various doses of diazepam on 24-hour toxicity of soman, sarin and VX in the rat

Antidotes (mg/kg)	Protective index		
	Soman	Sarin	VX
HI-6 (10) + atropine (10)	1.65	2.06	18.3
HI-6 (10 + atropine (10) + diazepam (0.5)	1.61	8.96*	36.3*
HI-6 (10) + atropine (10) + diazepam (0.5)	1.85	9.38*	38.6*
HI-6 (10) + atropine (10) + diazepam (2.5)	1.94	9.1*	36.4*
HI-6 (10) + atropine (10) + diazepam (5.0)	1.77	11.35*	31.0*

Antidotes were given by i.m. route, immediately before s.c. administration of poison.

* $P < 0.05$ compared to HI-6/atropine group

compounds used (although the midazolam effect was more prominent in VX treated rats). The results of preliminary experiments (not shown), with different doses of diazepam or midazolam (0.5, 1.0, 2.5 and 5.0 mg/kg), failed to show any protective effects in soman, sarin and VX treated rats. The efficacy of diazepam, atropine and oxime in rats poisoned by soman, sarin and VX is shown in Table 2. When rats received atropine and oxime the toxicity of soman, sarin and VX decreased 1.65, 2.06 and 18.3 times, respectively. The introduction of diazepam in the therapy led to further improvement of protective indices. The most pronounced protective effect was observed in rats poisoned by VX (up to 38.6) and sarin (up to 11.35). An almost negligible protective effect was shown in rats poisoned with soman. Table 3 shows the effect of midazolam, atropine and oxime on the 24-hour toxicity of soman, sarin and VX. Midazolam and diazepam in combination with atropine and oxime HI-6 offered the protection of similar magnitude to rats treated with VX and sarin (P.I. = 39.5:38.6 for VX and 13.67:11.35 for sarin). In contrast to VX and sarin, the toxicity of soman was only slightly reduced. The effect of flumazenil on benzodiazepine-induced potentiation of atropine/HI-6 antidotal efficacy in OP poisoned rats is shown in Table 4. The benzodiazepines-induced increment of protective indices (compared to the atropine/HI-6 group) was significantly diminished by the flumazenil treatment.

Table 3.

Effects of various doses of midazolam on 24-hour toxicity of soman, sarin and VX in the rat

Antidotes (mg/kg)	Protective index		
	Soman	Sarin	VX
HI-6 (10) + atropine (10)	1.65	2.06	18.3
HI-6 (10 + atropine (10) + diazepam (0.5)	1.61	9.13*	38.7*
HI-6 (10) + atropine (10) + midazolam (0.5)	1.5	13.67*	39.5*
HI-6 (10) + atropine (10) + midazolam (2.5)	1.85	11.71*	31.5*
HI-6 (10) + atropine (10) + midazolam (5.0)	1.66	9.09*	34.4*

Antidotes were given by i.m. route, immediately before s.c. administration of poison.

* $P < 0.05$ compared to HI-6/atropine group

Table 4.

The effect of flumazenil on benzodiazepine-induced potentiation of atropine/HI-6 antidotal efficacy in OP poisoned rats

Treatment (mg/kg)	Protective index	
	Sarin	VX
HI-6 (10) + atropine (10	2.1	18.3
HI-6 (10) + atropine (10) + diazepam (2.5)	9.1	36.4
HI-6 (10) + atropine (10) + diazepam (2.5) + flumazenil (10)	3.8*	22.09*
HI-6 (10) + atropine (10) + midazolam (2.5)	11.7	31.5
HI-6 (10) + atropine (10) + midazolam (2.5) + flumazenil (10)	3.5*	20.8*

Antidotes were given by i.m. route (flumazenil i.p.), immediately before s.c. administration of poison.
* $P < 0.05$ compared to rats treated with atropine (HI-6)benzodiazepines

DISCUSSION

Both benzodiazepines have a similar mechanism of action (gabaergic) but clinically, midazolam is used primarily as a hypnotic-sedative drug. In our experiments both benzodiazepines exerted a powerful anticonvulsive effect in soman, sarin and VX treated rats. Their effect was of equal magnitude. These results confirmed the finding of *Pieri and co-workers* (6) that diazepam and midazolam possessed a similar anticonvulsive activity in various kinds of chemically-induced convulsions. The lowest dose of midazolam and diazepam (0.5 mg/kg) provided a reliable protective effect in rats poisoned by sarin and VX. However, it must be noted that at higher dosage levels (2.5, 5 mg/kg) there was no further improvement of protective indices. This is in agreement with the results of *Miller and co-workers* (7) that a powerful pharmacological effect could occur even under the conditions of relatively low occupancy of benzodiazepine receptors (30–60%).

Considering the pharmacokinetics of diazepam ($t/2 = 42.4$ h) and the long-lasting persistence of its active metabolites in circulation diazepam could be expected to »cover« the period of intoxication by OP compounds better than midazolam. It could be speculated that a slightly better efficacy of midazolam compared to that of diazepam may be due either to the better absorption of midazolam from its site of injection, or to the fivefold greater affinity of this compound for the benzodiazepine receptors, as was shown by *Mohler* (8). Flumazenil is the first specific benzodiazepine receptor antagonist (9) with a short half-life (0.7–1.3 h). The limited duration of action (2–3 hours) is due to its rapid hepatic elimination (10). The ability of flumazenil to counteract, almost

equally, the beneficial effect of midazolam and diazepam in sarin and VX poisoned rats strongly suggests the important role of the gabaergic system during the initial stage of OP intoxication. Contrary to VX and sarin, the poisoning with soman, oxime-resistant organophosphate (11, 12) is still a serious therapeutic problem. Following its reaction with the enzyme AChE, soman undergoes a rapid chemical change (an ageing process) which makes ChE reactivation impossible (13). Besides that, a soman depot has been proposed (14). It is possible that some AChE that was reactivated in the initial stages of oxime therapy could be reinhibited by the soman appearing from the depot.

The present experiments show that none of the benzodiazepines tested reduced the toxic effect of soman in animals treated with atropine and oxime HI-6. These data support the concept that a significant benzodiazepine-oxime/atropine interaction might be achieved only in the presence of at least partially recovered AChE activity and cholinergic functions.

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Sažetak

ANTIKONVULZIVNI I ZAŠTITNI EFEKTI DIAZEPAMA I MIDAZOLAMA U TROVANJU VISOKOTOKSIČNIM ORGANOFOSFORNIM JEDINJENJIMA

Cilj istraživanja je bio da se međusobno uporede antikonvulzivni i zaštitni efekti diazepama i midazolama u pacova trovanih nervnim bojnim otrovima. U pacova trovanih somanom, sarinom i VX otrovom midazolam i diazepam ispoljavaju antikonvulzivne efekte približno istog intenziteta. Standardna antidotska terapija (atropin, oksim HI-6) smanjuje toksičnost somana, sarina i VX otrova 1,65, 2,06 i 18,3 puta. Uvođenjem benzodiazepina midazolama i diazepama u terapiju, zaštitni efekti standardnih antidota značajno se poboljšavaju. Primenjen uz standardne antidote midazolam je ispoljio nešto bolja zaštitna dejstva od diazepama a ovi efekti su registrovani posle primene i relativno malih doza benzodiazepina (0,5 mg/kg). Specifični antagonista benzodiazepinskih receptora (Flumazenil) gotovo u potpunosti poništava terapijske efekte primenjenih benzodiazepina. Navedeni podaci ukazuju na značajnu ulogu gabaergičkog sistema u trovanjima organofosfornim jedinjenjima, posebno tokom prve faze intoksikacije.

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Ključne reči: antikonvulzivi, antidoti, atropin, benzodiazepini, kemijski bojni otrovi, terapija trovanja, Flumazenil, oksim HI-6, sarin, soman, subletalne doze, VX.