Department of Pharmacology, Faculty of Pharmacy, Belgrade, Yugoslavia

The treatment of delayed polyneuropathy induced by diisopropylfluorophosphate in hens

R. M. Stepanović Petrović, M. Jokanović, M. Maksimović, N. Ugrešić and B. Bošković

This study was undertaken to examine the influence of atropine, oximes and benzodiazepine on organophosphate-induced delayed polyneuropathy (OPIDP) in hens, which were poisoned with diisopropylfluorophosphate (DFP). The birds were treated with a standard neuropathic dose of DFP (1.1 mg/kg, sc), which produced typical signs of OPIDP. The development of OPIDP was observed within the followings 22 days. All drugs were given subcutaneously (sc), intramuscularly (im) or intraperitoneally (ip), 20 min before the poison. The results obtained have shown that atropine (20 mg/kg, ip) only in combination with oxime TMB-4 (15 mg/kg, im) produced significant improvement of OPIDP symptoms in comparison with positive control. Clinical signs and symptoms of OPIDP in the group which was treated with atropine (20 mg/kg, ip), TMB-4 (15 mg/kg, im) and midazolam (2.5 mg/kg, im) were more improved than that in the presence of a combination of atropine and TMB-4. The results of these experiments have shown that it is possible to prevent the development of DFP-induced OPIDP in hens by treatment with atropine and TMB-4 or atropine, TMB-4 and midazolam when given before DFP.

1. Introduction

Organophosphorus (OP) compounds cause two major toxic effects. The first is the well-known acute neurotoxic effect during the cholinergic phase of OP poisoning. The second is called organophosphate-induced delayed polyneuropathy (OPIDP) usually manifested as distal motor polyneuropathy [1−4]. OPIDP is thought to be initiated by inhibition of more than 70% of nerve enzyme neuropathy target esterase (NTE) and intramolecular rearrangement of the phosphorylated NTE called aging [1, 3, 4]. Clinical signs and symptoms of OPIDP in humans and susceptible animal species (hen is the most sensitive) are incoordination, ataxia, spasticity and flaccid paralysis developing distally in the hind limbs and eventually spreading to the fore limbs [5−8]. These signs occur two to four weeks after OP dosing.

Previous studies have shown that prophylactic use of inhibitory phosphinates and related compounds [9], several corticosteroids [10], nicotinic acid derivatives [11, 12], verapamil [7, 13] and phenobarbital [14], partially or completely prevent the development of OPIDP. There no literature data about an effect of simultaneous applied anticholinergics, oximes and benzodiazepines on OPIDP in hens. Our previous results had shown that the anticholinergic atropine and the oxime TMB-4 were successful in decreasing the rate of aging of inhibited NTE *in vitro*. Thus we examined the influence of those drugs and others which were used for the treatment of neuronal disturbances such as benzodiazepines, on OPIDP in hens which were poisoned with diisopropylfluorophosphate (DFP).

2. Investigations and results

In all positive control groups of hens, clinical signs of OPIDP developed 8 days following administration of the poison. OPIDP progressed in severity over the next period until day 22, and at the end of experiment the effect could be graded as 5 (Tables 1, 2).

Following the administration of atropine (20 mg/kg, ip) and the oximes: diacetylmonoxime (DAM) (30 mg/kg, im), HI-6 (100 mg/kg, im) and pralidoxime (PAM-2) (100 mg/kg, im), there were no significant changes. But in the group treated with the combination of atropine and oxime trimedoxime (TMB-4) (15 mg/kg, im), (group V, Table 1), the signs of OPIDP were significantly improved and graded as 3 (Table 1).

In the experiments where we examined the influence of midazolam (25 mg/kg, im) in the presence of atropine (20 mg/kg, ip) and TMB-4 (15 mg/kg, im), on poisoned hens, the improvement of clinical evidence of OPIDP was significant and greater than that in the presence of combination of atropine (20 mg/kg, ip) and TMB-4 (15 mg/kg, im), compared to positive control (Table 2). Clinical signs and symptoms of OPIDP in the group, which was treated with atropine/TMB-4/midazolam combination (group III), were graded as 2 (Table 2).

Table 1: Effect of atropine, DAM, HI-6, PAM-2 and TMB-4 on DFP-induced development of OPIDP in hens

Group	Treatment	Mean ± SEM[a]	n[d]
I	Positive control[b]	5 ± 1	9
II	Atropine + DAM + DFP	4 ± 2	6
III	Atropine + HI-6 + DFP	5 ± 2	6
IV	Atropine + PAM-2 + DFP	5 ± 2	6
V	Atropine + TMB-4 + DFP[c]	3 ± 1	8

[a] Clinical evaluation was blindly performed on each hen daily for 22 days according to the 8-point scale [23], where 0 represents normal gait and 8 total paralysis
[b] Positive control hens were treated with atropine and physostigmine before dosing DFP in order to overcome acute effects of DFP poisoning
[c] $P < 0.05$ compared to the control
[d] N represents the number of hens in each group

Table 2: Effect of atropine, TMB-4 and midazolam on DFP-induced development of OPIDP in hens

Group	Treatment	Mean ± SEM[a]	n[d]
I	Positive control[b]	5 ± 2	9
II	Atropine + TMB-4 + DFP[c]	3 ± 1	8
III	Atropine + TMB-4 + midazolam + DFP[c]	2 ± 1	6

[a] Clinical evaluation was blindly performed on each hen daily for 22 days according to the 8-point scale [23] where 0 represents normal gait and 8 total paralysis
[b] Positive control hens were treated with atropine and physostigmine before dosing DFP in order to overcome acute effects of DFP poisoning
[c] $P < 0.05$ compared to the control
[d] N represents the number of hens in each group

3. Discussion

It has been shown that pralidoxime (PAM-2) and atropine have no effect on the development of delayed neurotoxic signs in chickens after poisoning with DFP [15]. In accordance with this, significant changes in OPIDP after applied combination of atropine and PAM-2 were not seen after poisoning with DFP. Similarly, we have shown that neither the combination of atropine and HI-6 nor the combination of atropine and DAM had any effect on OPIDP. On the contrary, only the combination of atropine and TMB-4 gave a significant improvement in clinical evidence of OPIDP. These findings were not unexpected since our previous *in vitro* investigation indicated that the combination of atropine and oxime TMB-4 was the most effective one in decreasing the rate of aging on DFP-inhibited NTE. The effect was concentration-dependent [16, 17]. A possible explanation for this effect of atropine and TMB-4 is that atropine alters the conformation of NTE as a protein, which makes TMB-4 approach to active site of NTE, and aging of NTE is retarded and decreased. In this way the clinical impression of OPIDP was improved.

In the present experiments a better protective effect was achieved with the former combination plus the benzodiazepine, midazolam. Similarly, Bokonjić [18] found a very good protection, upon acute poisoning with OP compound VX in rat, when applied a combination of atropine, oxime HI-6 and midazolam. He suggested that this combination, anticholinergic/oxime/benzodiazepine, had a good protective effect only under the conditions of at least partly available activity of acetylcholinesterase (AchE) and synaptic acetylcholine (Ach) function. In the light of this explanation, our good protective results with atropine/TMB-4/midazolam may be attributed to ceasing hyperactivity of the cholinergic system by atropine, decreasing aging of NTE by TMB-4, and stabilizing neuron membrane by midazolam via augmentation of the action of gamma-aminobutyric acid (GABA) [19, 20]. The remarkably good influence of midazolam in combination of atropine and TMB-4 on OPIDP could be explained otherwise. Bošković [21] has shown that diazepam increased the survival time of an atropine/TMB-4 combination even 35-fold in rats poisoned with soman. The author suggested that diazepam counteracted the "direct" toxic biochemical effect that soman exerted. Namely, soman produces an increase in cerebellar cGMP concentration, but diazepam decreases cerebellar cGMP concentration in rats or mice [22]. In the light of the antagonistic action of diazepam in soman poisoning, we can propose that midazolam might have a similar antagonistic effect on DFP-increase of cGMP concentration.

In conclusion, the results of these examinations have shown that atropine and TMB-4 or atropine, TMB-4 and midazolam decrease the neurotoxic effect of DFP. The improvement of clinical signs and symptoms of OPIDP is greater in the case of applying the combination of atropine, TMB-4 and midazolam than that in the presence of atropine and TMB-4 in comparison with positive control. Since this type of study has not been done before, the drug combination might be used as a possible protective tool in clinical conditions.

4. Experimental

4.1. Chemicals

Diisopropylfluorophosphate (DFP) and oximes: pralidoxime chloride (PAM-2), trimedoxime chloride (TMB-4) and HI-6 (purity >97%) were obtained from the Military Technical Institute, Belgrade, Yugoslavia. Diacetylmonoxime (DAM) was purchased from Fluka, Buchs, Switzerland. Atropine sulphate was supplied by Sigma, Poole, UK. Midazolam (Flormidal®) was obtained from ICN Galenika, Yugoslavia.

4.2. Experimental animals and treatments

All drugs were given subcutaneously (sc), intramuscularly (im) or intraperitoneally (ip), 20 min before poisoning of hens with DFP (1.1 mg/kg). The birds were observed daily for clinical signs of OPIDP during 22 days and the degree of walking impairments and ataxia were assessed according to an 8-point scale [23] where 0 represents normal gait and 8 total paralysis.

4.3. Delayed neurotoxicity experiments

In two kinds of experiments a positive control group of hens was treated with physostigmine (0.15 mg/kg, ip) and atropine (2 mg/kg, ip) to overcome the signs of acute poisoning.

4.3.1. Influence of oximes on OPIDP

There were five experimental groups (n = 6–9) of hens: the first group served as positive control for OPIDP and other groups were dosed with a combination of atropine (20 mg/kg, ip) and oximes: DAM (30 mg/kg, im), HI-6 (100 mg/kg, im), PAM-2 (100 mg/kg, im), and TMB-4 (15 mg/kg, im), respectively.

4.3.2. Influence of midazolam on OPIDP

There were three experimental groups (n = 6–9) of birds: the first group served as positive control for OPIDP, the second group was dosed with atropine (20 mg/kg, ip) and TMB-4 (15 mg/kg, im), and the third group was additionally dosed with midazolam (2.5 mg/kg, im).

4.4. Statistical analysis

Data are expressed as mean ± SEM. The statistical evaluation of the data was performed by the Student's t-test and the $p < 0.05$ was considered as statistically significant.

References

1 Johnson, M. K.: Rev. Biochem. Toxicol. **4**, 141 (1982)
2 Senanayake, N.; Karalliedde, L.: N. Engl. J. Med. **316**, 761 (1987)
3 Lotti, M.; Moretto, A.; Bertolazzi, M.; Peraica, M.; Fiorini, F.: Arch. Toxicol. **69**, 330 (1995)
4 Jokanović, M.; Maksimović, M.; Stepanović R. M.: Arch. Toxicol. **69**, 425 (1995)
5 Davis, C. S.; Richardson, R. J.; in: Spencer, P. S.; Schaumburg, H. H. (Eds.): Experimental and Clinical Neurotoxicology, p. 527. Williams & Wilkins, Baltimore 1980
6 Metcalf, R. L.: in Cranmer, J. M. Hixson, E. J. (Eds.): Delayed Neurotoxicity. Proceedings of the Delayed Neurotoxicity Workshop presented June 27–30, 1982 at the University of Illinois at Urbana-Champaign, p. 7., Intox Press, Little Rock 1984
7 El Fawal, H. A.; Correll, L.; Gay, L.; Ehrich, M.: Toxicol. Appl. Pharmacol. **103**, 133 (1990)
8 Ehrich, M.; Jortner, B. S.; Padilla, S.: Fund. Appl. Toxicol. **24**, 94 (1995)
9 Lotti, M.: Toxicology. **21**, 465 (1992)
10 Baker, T.; Drakontides, A. B.; Riker, W. F. Jr.: Exp. Neurol. **78**, 397 (1982)
11 Chambers, H. W.; Casida, J. E.: Toxicol. Appl. Pharmacol. **10**, 105 (1967)
12 Chambers, H. W.; Casida, J. E.: Toxicol. Appl. Pharmacol. **14**, 249 (1969)
13 El-Fawal, H. A. N.; Jortner, B. S.; Ehrich, M.: Toxicol. Appl. Pharmacol. **97**, 500 (1989)
14 Konno, N.; Katoh, K.; Yamaguchi, Y.; Yamauchi, T.; Fukushima, M.: Arch. Environ. Contam. Toxicol. **15**, 653 (1986)
15 Davies, D. R.; Holland, P.: Biochem. Pharmacol. **21**, 3145 (1972)
16 Stepanović, R. M.; Jokanović, M.; Maksimović, M.; Bošković, B.; Ugrešić, N.: Pharmacol. Res. **31**, Suppl, 147 (1995)
17 Jokanović, M.; Stepanović, R.M.; Maksimović, M.; Kosanović, M.; Stojiljković, M. P.: Toxicology Lett. **95**, 93 (1998)
18 Bokonjić, D.: Doctoral thesis, Belgrade, 1994
19 Costa, E.: Trends Pharmacol. Sci. October, 41 (1979)
20 Rang, H. P.; Dale, M. M. (eds.) in: Pharmacology, p. 632, Churchill Livingstone, London 1991
21 Bošković, B.: Fund. Appl. Toxicol. **1**, 203 (1981)
22 Lundy, P. M.; Magor, G. F.: J. Pharm. Pharmac. **30**, 251 (1978)
23 Roberts, N. L.; Farley C.; Philips, C.: Neurotoxicology. **4**, 263 (1983)

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Radica Stepanović Petrović, Ph.D.
Assistant Professor
Department of Pharmacology
Faculty of Pharmacy
Vojvode Stepe 450
11221 Belgrade
Yugoslavia